BBA 45718

STUDIES ON THE PROPERTIES OF CHEMICALLY MODIFIED ACTIN

II. TRINITROPHENYLATION

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SUMMARY

1. 4–10 amino groups of the 31 ε-amino groups of actin were trinitrophenylated by trinitrobenzene sulfonate, depending on the conditions of the reaction, and the characteristic properties of the trinitrophenylated actin were studied.

2. The effect of trinitrophenylation was more definite on the polymerizability of G-actin than on the depolymerization of the F-form.

3. The degree of actomyosin formation depended mainly on the pH of the trinitrobenzene sulfonate treatment.

4. The superprecipitation of synthetic actomyosin reconstituted from trinitrophenylated actin decreased more sharply when the actin was in the G-form than when it was in the F-form.

5. The myosin ATPase (ATP phosphohydrolase, EC 3.6.1.3) activation property of actin sharply decreased at a very low degree of trinitrophenylation in the presence of α-actinin. α-Actinin enhanced the ATPase activity of actomyosin reconstituted from control actin, but had no effect on the ATPase of actomyosins reconstituted from trinitrophenylated actins.

6. The ATPase activation by actin increased with the increasing degree of trinitrophenylation, when more than 1 mole amino groups per mole actin was trinitrophenylated.

INTRODUCTION

The effect of photooxidation, succinylation and nitration on the characteristic properties of actin was reported in a foregoing paper[1]. Here an account is given on the effect of trinitrophenylation.

According to TONOMURA and co-workers[2,3] trinitrobenzene sulfonate specifically trinitrophenylates some of the ε-amino groups of actin. If 1 mole ε-amino group per mole actin was trinitrophenylated, actin lost its capacity to form actomyosin without any loss of polymerizability[2].

Abbreviation: TNP-, trinitrophenyl-.

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MATERIALS AND METHODS

Materials and methods were generally the same as in the foregoing paper[1].

Tropomyosin free[4], normal 'Straub type' actin used in most experiments was purified according to BÁRÁNY *et al.*[5] and MOMMAERTS[6]. In some experiments α-actinin contamination was removed from the actin preparation according to EBASHI AND MARUYAMA[7].

Trinitrobenzene sulfonate was purchased from Sigma Chemical Co.

Actin was trinitrophenylated at 0° in G- or F-form, *i.e.*, in the presence or absence of 0.1 M KCl (the salt milieu used for polymerization). The solution contained 2 mg of protein per ml, 10 mM Tris–HCl buffer (pH 8.65 or 8.95) and 0.2 mM ATP. Free ATP was removed by Dowex-1 treatment[8] in some experiments. 0.75 mM trinitrobenzene sulfonate was added to the actin solutions. 5-ml aliquots were withdrawn in due times. KCl (final concentration 0.1 M) and maleic acid were added to the samples in order to polymerize G-actin and to stop further trinitrophenylation by lowering the pH to 7 (ref. 2). Therefore all the samples were in polymerized (F) form during the measurements of all the characteristic properties. G- or F-actin means only the state of the sample during trinitrophenylation. Samples were dialysed against 50 vol. of 0.1 M KCl–5 mM Tris–maleate buffer (pH 6.8) at 0° for 36 h with three changes of the dialysing solution.

The number of trinitrophenyl-(TNP-)lysine groups formed were calculated according to OKUYAMA AND SATAKE[9] on the basis of absorbance change at 346 mμ ($\varepsilon = 1.45 \cdot 10^4$).

RESULTS

The rate of trinitrophenylation depended on the presence or absence of 0.1 M KCl in the reaction mixture (Fig. 1). The ε-amino groups of F-actin were trinitro-

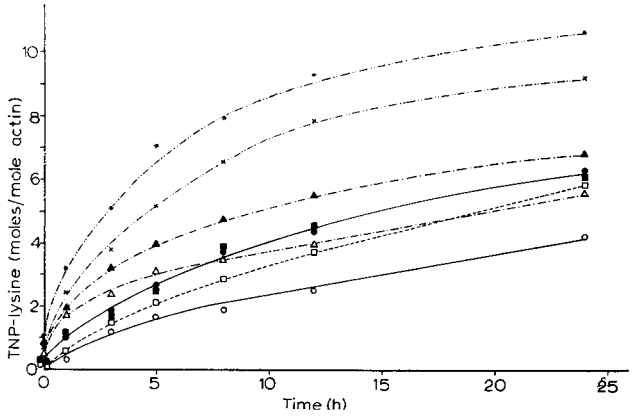

Fig. 1. The rate of trinitrophenylation of actin. Conditions of the reaction: ○—○, G-actin, ATP present, pH 8.65; □---□, G-actin, ATP present, pH 8.95; △–·–△, G-actin, ATP absent, pH 8.95; ●—●, F-actin, ATP present, pH 8.65; ■—■, F-actin, ATP present, pH 8.95; ▲–·–▲, F-actin, ATP absent, pH 8.65; ✱–··–✱, irreversibly depolymerized actin, 0.1 M KCl present, pH 8.65; ×–··–×, irreversibly depolymerized actin, KCl absent, pH 8.65; Mol. wt. of actin, 60000. For details of the procedure see MATERIALS AND METHODS.

phenylated at a higher rate than the samples in the G-form, under identical conditions. This effect is attributed to some general ionic effect of KCl and not to its property of polymerizing actin, as the effect could be observed if the actin was denaturated (irreversibly depolymerized) by the removal of the bound nucleotide by excessive dialysis against distilled water[10]. In the latter case the addition of KCl did not cause polymerization, but enhanced the rate of trinitrophenylation, which excluded the possibility that the difference between the G- and F-form of actin was the reason for the different reaction rates. The denaturation (irreversible depolymerization) of actin increased the rate of reaction. The absence of free ATP increased mainly the initial velocity of trinitrophenylation both in the G- and F-forms. The increase in the pH did not affect the rate of the reaction of F-actin, but the rate of the trinitrophenylation of G-actin slightly increased.

The effect of trinitrophenylation on the polymerizability of G-actin and on the depolymerization of F-actin was studied (Fig. 2). All viscosity measurements were carried out in the presence of 0.1 M KCl. Therefore G- and F-actin means only the state of the sample during the procedure of trinitrophenylation and not during the measurement of the characteristic properties.

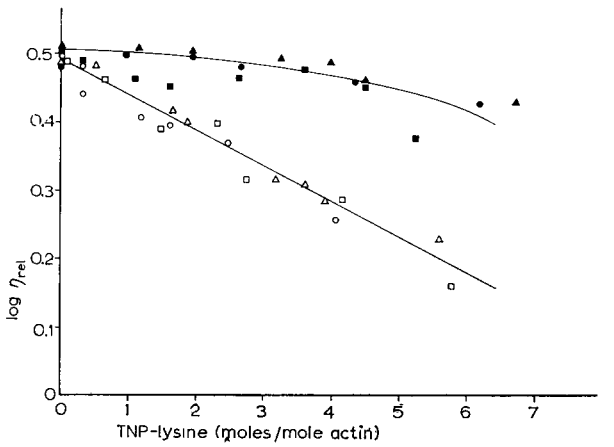

Fig. 2. Effect of trinitrophenylation on the viscosity of actin. Conditions of trinitrophenylation: ○, G-actin, ATP present, pH 8.65; □, G-actin, ATP present, pH 8.95; △, G-actin, ATP absent, pH 8.65; ●, F-actin, ATP present, pH 8.65; ■, F-actin, ATP present, pH 8.95; ▲, F-actin, ATP absent, pH 8.65. For details of the procedure see METHODS of the foregoing paper[1].

According to the results, the polymerizability of G-actin decreased to 50% of the control if 5 moles of ε-amino groups per mole actin were trinitrophenylated. F-actin depolymerized but very slightly in consequence of trinitrophenylation. The effect of trinitrophenylation did not depend on either the pH of the reaction or the presence of ATP.

Different results were obtained when the formation of actomyosin from trinitrophenylated actin was studied at high ionic strength, assessed by ATP sensitivity[11] (Fig. 3). The decrease of ATP sensitivity did not depend on whether actin was in the G- or the F-form during trinitrophenylation, it depended, however, on the pH of the reaction mixture and on the presence of ATP. At higher pH values and in the absence of ATP the ATP-sensitivity values decreased much more than at a lower pH and in

the presence of ATP. The F- and G-actin curves differed only in the absence of free ATP, the latter decreasing more sharply than the former one.

The superprecipitation of actomyosins reconstituted from differently trinitrophenylated actins depended on the state of actin during the reaction, and on the

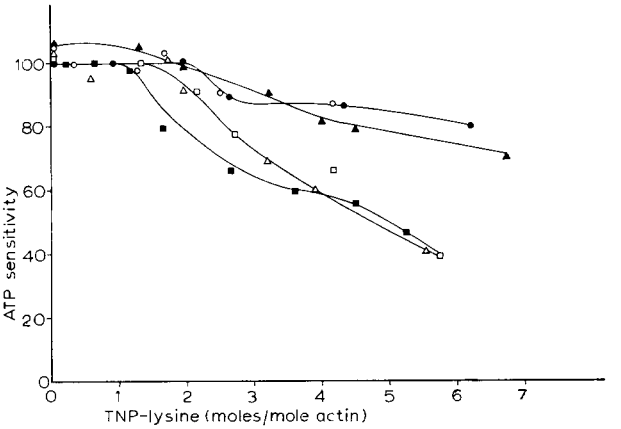

Fig. 3. Effect of trinitrophenylation of actin on the actomyosin formation at high ionic strength. Legends see Fig. 2. For details of the procedure see METHODS of the foregoing paper[1]. The decrease of ATP sensitivity means decreasing actomyosin formation since the viscosities before the addition of ATP diminish in the consequence of trinitrophenylation and the log η_{rel} values after the addition of ATP are always the sum of the log η_{rel} of the original actin and myosin solutions[22].

number of TNP-lysine residues formed (Fig. 4). Superprecipitation decreased to 50 % of the original value when 1.8 moles amino groups per mole actin were trinitrophenylated in the case of G-actin, while a similar decrease was observed with the F-form only if 4.5 moles ε-amino groups per mole actin were reacted. The superprecipitation values did not depend on the pH or on the presence of free ATP in the reaction mixture.

Not only the values of superprecipitation, which were calculated on the basis of turbidity change[12] (*i.e.*, the difference between absorbance at 546 mμ observed before

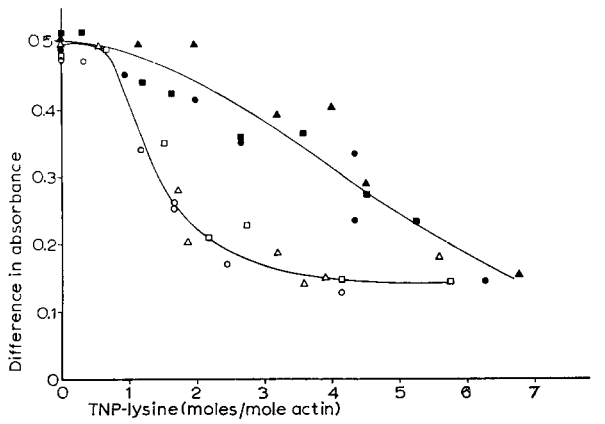

Fig. 4. Effect of trinitrophenylation of actin on the superprecipitation of synthetic actomyosin. Legends see Fig. 2. For details of the procedure see METHODS of the foregoing paper[1].

and after the addition of ATP), but the time needed for the onset of superprecipitation after the addition of ATP depended on the degree of trinitrophenylation. Three characteristic superprecipitation curves are presented in Fig. 5 together with the corresponding ATPase (actomyosin ATPase, Mg^{2+} present, measured on the basis of pH change[1]) curves which were measured simultaneously[1].

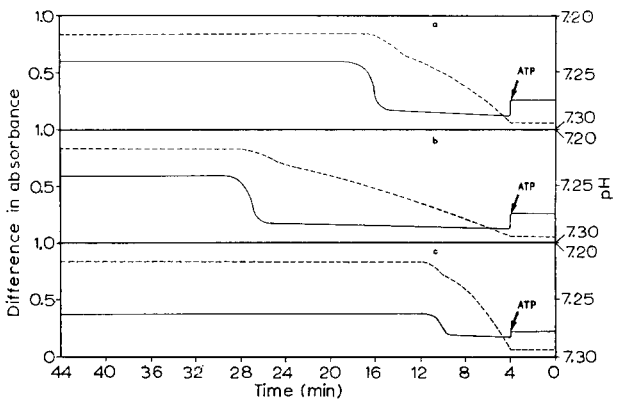

Fig. 5. Simultaneous superprecipitation and ATPase curves of synthetic actomyosins. For details of the measurement of ATPase activity and superprecipitation see METHODS of the foregoing paper[1]. Actomyosin reconstituted from: a, normal; b and c, trinitrophenylated actins. Degree of trinitrophenylation: b, 0.6 and c, 4.9 mole TNP-lysine per mole actin. Conditions of trinitrophenylation: F-actin 0.2 mM ATP present, pH 8.65. Conditions of the enzymic assay: 0.9 mg of myosin per ml, 0.15 mg actin per ml, 1 mM ATP, 1 mM $MgCl_2$, 20 mM Tris-maleate buffer (pH 7.3), 23°. ———, absorbance; – – –, pH.

According to the results there is a close relationship between the remaining ATP concentration in the reaction mixture and the onset of superprecipitation. This seems to support the assumption of EISENBERG AND MOOS[13] who have suggested that superprecipitation takes place only if the decrease of ATP concentration reaches a 'threshold' level. Therefore the ATPase activity is in inverse ratio to the time needed for the onset of superprecipitation. If the ATPase activity is high the time needed for the onset of superprecipitation is small and *vise versa*.

According to Fig. 5b the trinitrophenylation of relatively few ε-amino groups (0.6 mole per mole actin) did not affect the value of superprecipitation, but greatly diminished the ATPase activity and increased the time needed for the onset of superprecipitation. On the other hand the trinitrophenylation of additional ε-amino groups (Fig. 5c) decreased the superprecipitation, enhanced the ATPase activity, and diminished the time necessary for the onset of superprecipitation.

One may assume that the reason for the decrease of the ATPase activity at a low degree of trinitrophenylation results from the reaction with some other protein present as a contamination in the actin preparations. The presence of α-actinin in the 'Straub type' actin preparations is well known[7] and according to MARUYAMA[14] α-actinin slightly activates actomyosin ATPase. We reexamined the effect of α-actinin on the ATPase activity of actomyosin reconstituted from α-actinin free actin (Fig. 6) and found a great activation. When the ATPase activity of actomyosins reconstituted from α-actinin-free trinitrophenylated actins was measured (Fig. 7) no decrease in activity was found at a low degree of trinitrophenylation. Extra α-actinin activated

the ATPase activity of actomyosin reconstituted from normal actin, but it did not affect the ATPase activity of the actomyosins reconstituted from the trinitrophenylated actins.

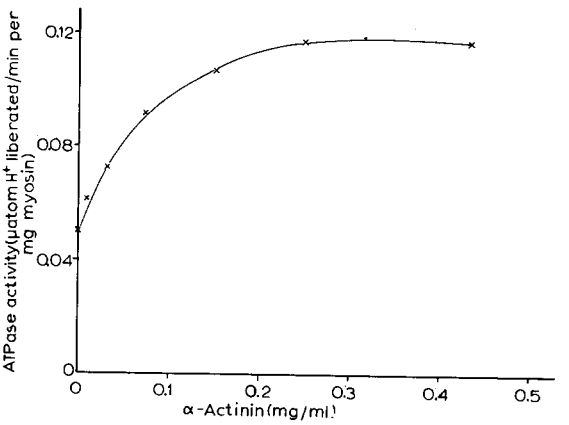

Fig. 6. Effect of α-actinin on the ATPase activity of actomyosin reconstituted from α-actinin-free actin. Details of the procedure see METHODS of the foregoing paper[1].

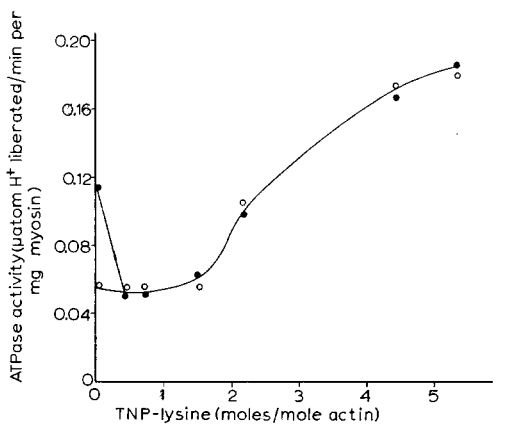

Fig. 7. Effect of trinitrophenylation of actin on the actomyosin ATPase in the presence and absence of α-actinin. Conditions of trinitrophenylation see Fig. 5. ●, α-actinin, 0.27 mg/ml; ○, α-actinin absent. For details of the procedure see METHODS of the foregoing paper[1].

The α-actinin activation of the actomyosin ATPase seems to be very sensitive to trinitrophenylation of actin, because it was completely lost when 0.5 mole lysine per mole actin was trinitrophenylated. This great lability was discussed already in the foregoing paper[1]. The presence or absence of α-actinin did not affect the increase in the actomyosin ATPase with the increasing number of ε-amino groups trinitrophenylated in the actin molecule. One could assume that the incomplete removal of trinitrobenzene sulfonate from trinitrophenylated actin samples by dialysis is the reason for the observed increase in the ATPase activity, as the activating effect of trinitrobenzene sulfonate on myosin ATPase in the presence of Mg^{2+} is well known[15]. This possibility, however, is excluded, because upon denaturation of the trinitro-

phenylated actins by acidifying and reneutralizing the samples, no activation of the myosin ATPase was found.

DISCUSSION

TONOMURA, TOKURA AND SEKIYA studied the effect of trinitrophenylation on the polymerizability and actomyosin formation of actin[2]. According to their results, when 1 lysine residue per mole actin was trinitrophenylated actomyosin formation was completely lost without any effect on the polymerizability of the protein. Our results are at variance with TONOMURA's because actomyosin formation decreased only by 50 % when 6-ε-amino groups were trinitrophenylated (Fig. 3), at pH 8.95 (even less at pH 8.65) and only in the case of F-actin at pH 8.95 was the decrease of ATP sensitivity greater than the change of polymerizability or depolymerization. One possible reason for this discrepancy is that actomyosin formation was calculated on the basis of ATP sensitivity (*i.e.*, viscosity measurements) in our studies and on the basis of light scattering measurements in the experiment of TONOMURA, TOKURA AND SEKIYA.

There is some uncertainty in the literature concerning the effect of α-actinin on the actomyosin ATPase. EBASHI *et al.* did not find any activation[16], MARUYAMA found a very small activation[14], whereas according to SERAYDARIAN, BRISKEY AND MOMMAERTS[17] there is, under strictly limited conditions, considerable activation of actomyosin ATPase. Our results presented in Fig. 6 show a two-fold activation of actomyosin ATPase which seems to support the data of the latter authors (the conditions of the ATPase test were very similar to theirs).

The most unexpected result is the increasing activation effect of actin on myosin ATPase with the increasing degree of trinitrophenylation (Figs. 5c and 7) both in the presence and absence of α-actinin. Similar results were obtained by succinylation[1] and acetylation*. In these cases, however, when a greater number of amino groups were modified (approx. 7 amino groups per mole actin) the activation effect decreased, *i.e.*, a maximum curve was obtained. One can find three possible explanations to this activation phenomenon: (1) Change in the actin molecule itself (conformational change or the modification of the active center) is the reason for the increasing activation. (2) There is some inhibitory factor present in the actin samples and this factor loses its activity in consequence of trinitrophenylation. (3) The inhibitor displays its effect through actin and actin loses its sensitivity to the factor with the growing number of ε-amino groups trinitrophenylated. These two latter assumptions are supported by the fact that a myofibrillar protein factor acting as inhibitor on the Mg^{2+}-activated ATPase[20,21] of actomyosin was found. It was impossible, however, to show the presence of this factor in actin preparations. Therefore further experiments are needed to elucidate this problem.

All the characteristic properties of actin are altered upon trinitrophenylation, but the changes ensue relatively independently. Actomyosin completely lost its α-actinin sensitivity when 0.5 amino group per mole actin were trinitrophenylated without any change in the other characteristic properties studied. When 4.5 ε-amino groups per mole actin were trinitrophenylated (F-actin, pH 8.65 in the presence of

* Unpublished results of the author.

ATP) superprecipitation decreased to about 50 %, actomyosin formation and viscosity to about 90 %, but ATPase activation increased to about 200 % of the control. The results presented here seem to support the assumption that different independent active centers are responsible for the characteristic properties of actin[1,18,19]. However, we can not exclude the possibility that the above mentioned changes are the consequence of some conformational modifications of the molecule. Trinitrophenylation decreases the number of positive charges of actin and the incorporation of the relatively large trinitrophenyl residue can disturb by itself the original conformation. Therefore, as in the case of the conclusions of the foregoing paper[1] we can not judge with certainty whether the amino groups reacted are actually taking part in the active centers or else are needed for the maintenance of the native conformation of actin.

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