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REACTION OF MYOSIN WITH SALICYLALDEHYDE

II. EFFECT OF SALICYLALATION ON THE ATPase ACTIVITY OF MYOSIN

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SUMMARY

The effect of salicylalation on the biological properties of myosin was studied.

1. The ATPase activity of myosin is affected by salicylalation if the treatment is carried out at higher pH than 6.5. The Mg^{2+}-activated ATPase shows a maximal curve with 250–380% maximal activation when 25–70 moles of salicylaldehyde are bound per mole of myosin. The EDTA-activated ATPase decreases with increasing salicylalation. Ca^{2+}-activated ATPase shows a small increase with increasing salicylalation.

2. Less salicylaldehyde is bound if the treatment is carried out in the presence of ATP, while that of PP_i does not affect the degree of salicylalation. The enzymic properties of myosins salicylalated in the presence of ATP or PP_i are not different from those of the samples treated in their absence.

3. Salicylalation decreases ATP sensitivity of ATPase and superprecipitation of actomyosins reconstituted from salicylalated myosins only if more than 50 moles of salicylaldehyde are bound per mole myosin.

INTRODUCTION

It was shown in the previous paper[1] that salicylaldehyde specifically reacts with the lysyl residues of myosin. Since it was found in the investigations of TONOMURA and co-workers[2–4] and of FÁBIÁN AND MÜHLRAD[5] that the trinitrophenylation of the ε-amino groups of myosin by 2,4,6-trinitrobenzenesulphonate (TBS) altered the enzymic properties of myosin and the alteration was observed to be markedly different from that caused by the blocking of the sulphydryl groups of myosin, it seemed of interest to study the changes in enzymic activity caused by the modification of the lysyl residues with another amino group reagent.

METHODS

The preparation of myosin and the treatment with salicylaldehyde, the determination of bound salicylaldehyde and protein concentrations were performed as described in MATERIALS AND METHODS in the previous paper[1].

Abbreviation: TBS, 2,4,6-trinitrobenzene sulphonate.

The ATPase activity of myosin was measured in a solution containing 1 mg of myosin per ml, 4 mM ATP, 50 mM Tris–maleate buffer (pH 7.6) and alternatively 5 mM EDTA, 5 mM $CaCl_2$ or 2 mM $MgCl_2$. Also present was 0.5 or 0.05 M KCl. The measurements were carried out on samples of 2 ml at 22°. Incubation was terminated by the addition of 2 ml of 10 % trichloroacetic acid. P_i was measured by the method of FISKE AND SUBBAROW[6]. The ATPase activity was evaluated as μmole P_i per mg of myosin per min. The incubation time was chosen so as to obtain a decomposition of the terminal phosphate of ATP to less than 25 %.

The ATPase activity of synthetic actomyosin was measured in a solution containing 0.8 mg of myosin per ml, 0.132 mg of actin per ml, 2 mM ATP, 2 mM $MgCl_2$ and 10 mM Tris–maleate buffer (pH 7.4) at 0°.

The superprecipitation was measured by the method of EBASHI[7] at 660 mμ with a Spectromom 360 spectrophotometer at room temperature. The conditions of the test were 0.8 mg of myosin, 0.066 mg of actin per ml, 0.1 mM ATP, 5 mM Tris–maleate buffer (pH 7.4) and 2 mM $MgCl_2$. Superprecipitation was evaluated from the difference between the values of the absorbance measured before, then 3 min after, the addition of ATP.

The actomyosin formation at high ionic strength was established from the measured ATP sensitivity. The measurements were carried out in an Ostwald viscometer using the method of BÁRÁNY *et al.*[8] under the following conditions: 1 mg of myosin per ml, 0.33 mg of actin per ml, 0.5 M KCl–0.02 M borate buffer (pH 7.4) at 0°.

RESULTS

The ATPase activities of myosins salicylalated at pH 6.5, 7.6, 8.0 and 9.0 were measured alternatively in the presence of Mg^{2+}, Ca^{2+} or EDTA each time in the presence of 0.5 M KCl. At pH 8 an additional run was made in the presence of 0.05 M KCl. The three types of the ATPase activity curve measured as a function of salicylalation at various pH's are shown in Fig. 1.

The ATPase activity curves (Fig. 1) measured in the presence of Mg^{2+} show maxima as a function of salicylalation. The maxima of the curves are 25–70 moles of bound salicylaldehyde per mole of myosin and appear at higher degrees of salicylalation for higher values of pH of the treatment. The maximal values of activation lie between 250 and 380 %. In the sample treated by salicylaldehyde at pH 6.5 the activation was hardly appreciable and also much less, not more than 13 moles of salicylaldehyde being bound per mole of myosin (see also Fig. 10 of the previous paper[1]). It seems that the very poor activation cannot be related to the decrease in the complex formation at pH 6.5, since at pH 7.6 at the same degree of salicylalation (13 moles of salicylaldehyde bound per mole of myosin) the activation was measured as approx. 200 %. It seems more probable that the lysyl residues having some role in the ATPase activity do not react with salicylaldehyde at pH 6.5.

The maxima of the Ca^{2+}-activated ATPase as a function of salicylalation were relatively small as compared with those measured in the presence of Mg^{2+}, and at higher degrees of salicylalation the activity was lower than that measured on the non-treated control. This may be due to unspecific conformational changes caused by the binding of numerous moles of salicylaldehyde to the myosin molecule.

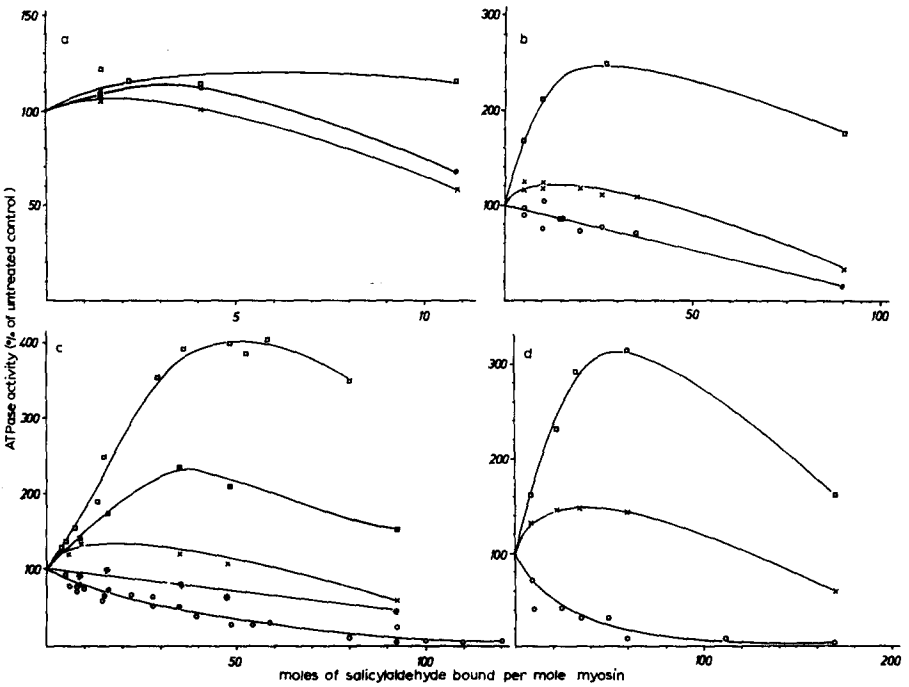

Fig. 1. The ATPase activity of myosin salicylalated at different pH's. Myosin was salicylalated in and dialysed against a solution containing 0.5 M KCl and 20 mM Tris–maleate buffer (a and b), or 20 mM borate buffer (c and d). a. pH 6.5. b. pH 7.6. c. pH 8.0. d. pH 9.0. For other conditions of the salicylalation see MATERIALS AND METHODS in the previous paper[1]. Conditions of the enzymic assay: 1 mg of myosin per ml; 50 mM Tris–maleate buffer (pH 7.4); 4 mM ATP; 0.5 (□, ○, ×) or 0.05 (■, ●, ✱) M KCl; and alternatively 5 mM EDTA (○, ●), 5 mM $CaCl_2$ (×, ✱) or 2 mM $MgCl_2$ (□, ■). Control (100%) activities (μmoles P_i per mg myosin per min): a. $CaCl_2$, 0.29; EDTA, 0.51; $MgCl_2$, 0.0041. b. $CaCl_2$, 0.25; EDTA, 0.60; $MgCl_2$, 0.0044. c. 0.5 M KCl: $CaCl_2$, 0.22; EDTA, 0.70; $MgCl_2$, 0.0054; 0.05 M KCl: $CaCl_2$, 0.46; EDTA, 0.14; $MgCl_2$, 0.0093. d. $CaCl_2$, 0.17; EDTA, 0.82; and $MgCl_2$, 0.0055.

The ATPase activity measured in the presence of EDTA, *i.e.* K^+-activated ATPase[9], markedly decreases with increasing salicylalation.

The measurements of ATPase activities at low ionic strength of the sample treated by salicylaldehyde at pH 8 (Fig. 1c) showed in each of the curves, for activity against salicylalation, lower values than those measured at high ionic strength.

The effect of the presence of magnesium ATP and magnesium pyrophosphate on the salicylalation of myosin was also studied since it was observed by TONOMURA, *et al.*[10], as well as by FÁBIÁN AND MÜHLRAD[5], that in the presence of either compound less lysyl residues were trinitrophenylated than in their absence. A similar phenomenon was also observed by BÁRÁNY *et al.*[11] on the reaction of myosin with 1-fluoro-2,4-dinitrobenzene. As apparent from Fig. 2, the presence of ATP reduced the degree of salicylalation, but PP_i did not appreciably affect the reaction between salicylaldehyde and myosin. In this experiment an additional control sample was used which was treated by salicylaldehyde in the presence of the same magnesium concentration as that of the magnesium ATP or magnesium pyrophosphate test samples. No difference was observed between the reaction yields of the additional and the usual (see MATERIALS AND METHODS in the previous paper[1]) control samples.

The K^+-activated ATPase (ATPase measured in the presence of EDTA) of myosins salicylalated in the presence of magnesium ATP, magnesium pyrophosphate or Mg^{2+} showed the same dependence on salicylalation as the control sample treated by salicylaldehyde in the absence of these ingredients (Fig. 3). The Mg^{2+}-inhibited ATPase curves are also very similar to the control curve if magnesium pyrophosphate or Mg^{2+} are present during the treatment with salicylaldehyde. Higher activation at a lower degree of salicylalation was observed only in the presence of magnesium ATP during the treatment.

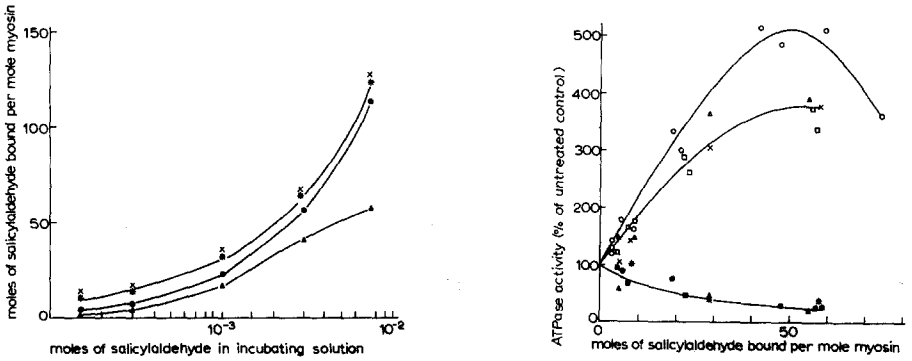

Fig. 2. Effect of ATP and PP_i on the salicylalation of myosin. Conditions of salicylalation: Both incubating and dialysing solutions: 0.5 M KCl and 0.1 M borate buffer (pH 8); other constituents of incubating solution: none (×); 6 mM $MgCl_2$ (✱); 6 mM $MgCl_2$ and 6 mM sodium pyrophosphate (●); 6 mM $MgCl_2$ and 6 mM ATP (▲). For dialysing solution: none (×); 2 mM $MgCl_2$ (✱); 2 mM $MgCl_2$ and 1 mM sodium pyrophosphate (●); 2 mM $MgCl_2$ and 1 mM ATP (▲). For other conditions see MATERIALS AND METHODS in the previous paper[1].

Fig. 3. ATPase activity of myosin salicylalated in the presence of ATP and PP_i. For conditions of salicylalation see legend to Fig. 2. Salicylalation was carried out in the presence of: ATP and Mg^{2+} (○, ●); PP_i and Mg^{2+} (□, ■); Mg^{2+} (△, ▲); no extra constituent (×, ✱). ATPase activity measured in the presence of 5 mM EDTA (●, ■, ▲, ✱) or 2 mM $MgCl_2$ (○, □, △, ×). Control (100%) activities: ●, 0.31; ■, 0.40; ▲, 0.34; ✱, 0.64; ○, 0.0048; □, 0.0044; △, 0.0039; ×, 0.0043 μmole P_i per mg myosin per min. The control EDTA-activated ATPases of those samples that were treated in the presence of Mg^{2+} (●, ■, ▲) were low because of the relatively high Mg^{2+} concentration present during the measurement of ATPase.

The effect of complex formation with salicylaldehyde on the characteristic properties of actomyosin synthesized from salicylalated myosin was also studied. The experimental data on ATPase activity, actomyosin formation (evaluated from the measured ATP sensitivity) and superprecipitation are listed in Table I. The actomyosin ATPase measured in the presence of Mg^{2+} at low ionic strength did not change on salicylalation when less than 46.5 moles of salicylaldehyde were bound per mole of myosin. At higher degrees of salicylalation a sharp decrease in actomyosin ATPase was observed which may be due to the unspecific conformation change taking place on the introduction of a large number of salicylaldehyde residues into the myosin molecule. The percentage activation of ATPase by actin decreased even on the addition of small amounts of salicylaldehyde because of the activating effect of salicylalation on myosin ATPase in the presence of Mg^{2+} (Table I, Column 2). (Activation by actin was evaluated as actomyosin ATPase/myosin ATPase × 100. Therefore, if the myosin ATPase increases without alteration of actomyosin ATPase, the activation by actin decreases.) A similar phenomenon was observed by FÁBIÁN AND MÜHLRAD[5]

TABLE I

THE CHARACTERISTIC PROPERTIES OF ACTOMYOSIN RECONSTITUTED FROM SALICYLALATED MYOSIN

For detailed conditions of enzymic assays, determinations of ATP sensitivity and superprecipitation see METHODS. Myosin ATPase was measured under the same conditions as actomyosin ATPase. Percentage activation by actin = actomyosin ATPase/myosin ATPase × 100.

Moles of salicylaldehyde bound per mole myosin	*Myosin ATPase*	*Actomyosin ATPase*	*Percentage activation by actin*	*ATP sensitivity*	*Superprecipitation*
	1	*2*	*3*	*4*	*5*
—	0.0234	0.227	970	160	0.61
3.2	0.0314	0.246	784	154	0.57
5.3	0.0340	0.249	734	158	0.54
13.7	0.0378	0.239	632	156	0.61
46.5	0.0340	0.109	466	143	0.36
82.6	0.0192	0.058	300	133	0.35
122.8	0.0058	0.003	52	80	0.00

for trinitrophenylated myosin[5]. Actomyosin formation and superprecipitation showed a similar decrease to that of actomyosin ATPase when more than 46.5 moles of salicylaldehyde were bound per mole of myosin.

DISCUSSION

The effect of the blocking of amino groups with salicylaldehyde on the enzymic properties of myosin is comparable to that observed on the blocking of these groups with TBS[2–5]. In both instances the Mg^{2+}-inhibited ATPase of myosin showed activation, whereas the Ca^{2+}-activated ATPase was left almost unchanged by the blocking (Figs. 2, 3 and 4, and ref. 5). In contrast, the blocking of sulphydryl groups results in the activation of both the Ca^{2+}-activated and Mg^{2+}-inhibited ATPase[12–16]. The K^+-activated ATPase of myosin decreases on any modification of myosin[3,12,14,5,17,18] and hence also on the blocking of sulphydryl or amino groups.

The other biological properties of myosin, such as the actomyosin formation, the Mg^{2+}-activated ATPase and the superprecipitation of actomyosin synthesized from the modified myosin, are also affected in almost the same way by the modification with salicylaldehyde as by that with TBS[5]. The effect of trinitrophenylation on the myosin ATPase is assumed to be due to the removal of the positive charges of one or some lysyl residues located in or near to the active site of the molecules[5].

The differences which can nevertheless be observed between the effects of trinitrophenylation and salicylalation on the enzymic properties suggest that trinitrophenylation more specifically affects the lysyl residues having some role in the ATPase activity. This would explain the observation that the activation of the Mg^{2+}-inhibited ATPase of myosin is 2000 %, if approx. 5 lysyl residues are trinitrophenylated per mole of myosin[5], as compared with an activation of 380 % if approx. 50 lysyl residues are salicylalated per mole of myosin.

The effect of the presence of the substrate is also different in the two cases. If ATP is present during trinitrophenylation the lysyl residues having some role in the ATPase activity are not modified since the change in the enzymic properties is much

less apparent than in the absence of the substrate. On the other hand the maximum activation of the Mg^{2+}-inhibited ATPase is observed at a lower degree of salicylalation in the presence than in the absence of ATP. The different effects of the two reagents in the presence of the substrate can be explained if one attributes to the lysyl residues having some role in the ATPase activity a higher affinity for TBS than the other lysyl residues of myosin. On the addition of ATP and Mg^{2+}, however, a conformational change takes place[11,18,20] by which the higher affinity of these specific lysyl residues is levelled off. The lysyl residues in question, however, have no higher affinity for salicylaldehyde and therefore the addition of the substrate, which equalizes the affinities of the residues for TBS, does not specifically affect the salicylaldehyde reaction of the lysyl residues involved in the ATPase activity of myosin.

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