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# COMPARATIVE STUDIES ON AMINO AND THIOL GROUPS IN MYOSINS FROM DIFFERENT SOURCES

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## Summary

Myosins from rabbit white and red skeletal, rabbit heart, fish skeletal and chicken gizzard muscles, as well as from human platelets were subjected to trinitrophenylation by trinitrobenzene sulfonate and alkylation by *N*-ethyl maleimide which affected their amino and thiol groups, respectively.

The blocking of amino groups was carried out in the presence or in the absence of Mg · ADP and was followed both spectrophotometrically and enzymatically. Essential amino groups, whose modification thoroughly changes the enzymic characteristics of myosin, were found in heart and in all skeletal muscle myosins but were absent in myosins from chicken gizzard muscle and from human platelets. The reaction of these amino groups was highly retarded in the presence of Mg · ADP.

Alkylation of thiols led to loss of the $K^+$-activated ATPase (ATP phosphohydrolase, EC 3.6.1.3) in all myosins. However, the rate of loss of activity varied from one myosin to another and, for a given myosin, was affected by the presence of nucleotides and by the value of the ionic strength. The change in $Ca^{2+}$-activated ATPase activity (ATP phosphohydrolase, EC 3.6.1.3) on alkylation was influenced by the presence of Mg · ADP during the reaction. In the absence of this nucleotide, the $Ca^{2+}$-ATPase activity increased and reached a plateau as a consequence of modification. The extent of activation largely depended on the origin of the myosin. When alkylation was carried out in the presence of Mg · ADP, the $Ca^{2+}$-ATPase activity as a function of time exhibited a maximum but the descending part of the curve was absent in myosins from heart and gizzard muscles.

## Introduction

The best characterized groups which are involved in the ATPase (ATP phosphohydrolase, EC 3.6.1.3) of white skeletal muscle myosin are thiol [1—3] and

amino groups [4—7]. On blocking the $SH_1$ thiol, which is characterized by high reactivity towards almost all -SH reagents, the $K^+$-activated ATPase activity is abolished while the $Ca^{2+}$- and $Mg^{2+}$-mediated ATPase of myosin is strongly activated [3]. On reaction of the $SH_2$ thiol with -SH reagents which, in the case of MalNEt, can be performed only in the presence of nucleotides after the blocking of $SH_1$, the $Ca^{2+}$-activated ATPase of myosin is also lost [2,3]. The effect of blocking the so-called essential amino group, which is localized in or near the active site of rabbit white skeletal muscle myosin is to abolish the $K^+$-activated, to decrease the $Ca^{2+}$-activated and to strongly increase the $Mg^{2+}$-mediated ATPase [4,5].

Since physiological, immunological and structural studies showed [8—13] that remarkable differences exist between different myosins and as practically all the work reported up till now on -SH and $-NH_2$ groups of the active site of this protein were associated with myosin obtained from rabbit white skeletal muscle, we decided to carry out a systematic comparative study of these functional groups in myosins from different sources. Profound differences were observed on comparing different myosins with respect to the reactivity of the -SH and $-NH_2$ groups, to the effect of chemical modification on the various ATPase activities and as regards the effect of ionic strength and temperature, as well as the presence of Mg · ATP or Mg · ADP, on the modification reaction. Such differences may reflect on the variation of the detailed mechanism of ATP splitting by myosins in different cells or cell organelles.

## Experimental Procedure

*Chemicals.* All chemicals used were reagent grade. ATP, ADP and $N_3BzSO_3$ were obtained from Sigma.

*Preparation of proteins.* White skeletal muscle myosin was prepared by the method of Azuma and Watanabe [14] using rabbit white back muscle. Rabbit red skeletal, rabbit heart, chicken gizzard (smooth) and fish (the electric eel, *Electrophorus electricus*) muscle myosins were prepared according to the method of Bárány [15]. Isolation of human platelet myosin, which was a generous gift from Dr. I. Cohen, was performed by the method of Pollard et al. [16].

*Trinitrophenylation of the amino groups of myosin.* $N_3BzSO_3$ treatment of myosin was carried out essentially as described earlier [5]. $N_3BzSO_3$ (final concentration 83 $\mu$M) was added to a solution containing 1.6 mg/ml myosin in 0.6 M KCl, 50 mM Tris/acetate buffer pH 7.4 and in some cases also 1.5 mM ADP and 2 mM $MgCl_2$. The reaction mixture was incubated at 25° C in a thermostatically temperature-controlled Gilford spectrophotometer cell where the reaction was followed on the basis of absorbance change at 345 nm. The number of trinitrophenylated amino groups was evaluated from the absorbance change ($\Delta\epsilon_{345}$ = 14 500) according to Okuyama and Satake [17]. In due time, aliquots were withdrawn and the ATPase activity was measured.

*Alkylation of thiol groups of myosin.* Alkylation was carried out by MalNEt. Two methods were employed.

The first method was used for the determination of the extent of changes in both $K^+$- and $Ca^{2+}$-dependent ATPases as a result of the alkylation of the thiol groups of myosin. Alkylation was performed by the addition of MalNEt (final

concentration 150 μM) to a solution containing 1.6 mg/ml myosin in 0.6 M KCl, 10 mM Tris/acetate buffer pH 7.4, either in the presence or in the absence of 2.5 mM ADP and 5 mM $MgCl_2$. Incubation was carried out at 25°C in the presence of Mg · ADP and at 0°C in its absence. Aliquots were taken in due course and their ATPase activities immediately measured after addition of 0.1 mM dithiothreitol in order to prevent further alkylation.

The second method was used in order to evaluate the rate of inactivation of the $K^+$-dependent ATPase on blocking the essential -SH groups as described by Watterson and Schaub [18]. For this purpose, alkylation was performed by the addition of MalNEt at different concentrations (5—20 μM) to 0.15 mg/ml myosin in 10 mM Tris/acetate buffer pH 7.4 under conditions given in the text. After 4.5 min incubation at 25°C, the $K^+$-activated ATPase activity of the samples was immediately measured in the presence of 0.1 mM dithiothreitol. The rate of inactivation was expressed as the percentage loss in activity per μM MalNEt.

*ATPase assay.* ATPase activity, expressed as μmol $P_i$/min per mg of myosin, was measured in 4 ml of solution containing 50—100 μg of protein, 1 mM ATP, 20 mM Tris · HCl buffer, pH 8.0 and either 2 mM $MgCl_2$/0.4 M KCl or 5 mM $CaCl_2$/0.6 M KCl or 5 mM EDTA/0.6 M KCl for the $Mg^{2+}$-, $Ca^{2+}$- and $K^+$-dependent ATPase activity determinations, respectively. The time of incubation was so chosen that less than 15% of the ATP present was hydrolyzed. Incubation was terminated by the addition of 0.75 ml of 1.65 M sulfuric acid and 0.0135 M ammonium molybdate. As reported by Bárány et al. [19], myosin does not precipitate, and therefore does not interfere with the determination of $P_i$ by the method of Fiske and SubbaRow [20], if its concentration is less than 25 μg/ml.

*Protein determination.* This was measured by the biuret method of Gornall et al. [21].

## Results

The reaction of amino groups of different myosins with $N_3BzSO_3$ in the presence and in the absence of Mg · ADP was followed spectrophotometrically. The course of trinitrophenylation are given in Fig. 1. The reaction curves consisted of two phases in all the cases studied. The rate of reaction in the initial faster phase depended on the presence of Mg · ADP for all skeletal and for rabbit heart myosins while no such dependence could be observed with smooth muscle and platelet myosins.

The velocity of reaction at the slower phase could be evaluated from the 20—40 min region of the curves, after the termination of the fast reaction. From this value the initial velocity (at $t$ = o) of the slow-reacting amino groups ($v_2$) could be obtained by taking into account the change in concentration of $N_3BzSO_3$ and of ε-amino groups during the first 20 min of the reaction. From the initial slopes of the curves in Fig. 1 we derived the initial velocity ($v$) of the overall process. The initial velocity of the fast reacting amino groups ($v_1$) was calculated by subtracting the velocity ($v_2$) of the slowly reacting groups from the initial velocity of the overall process ($v$) (Table I). The velocities of the slow-reacting groups were found to be nearly identical for all myosins studied,

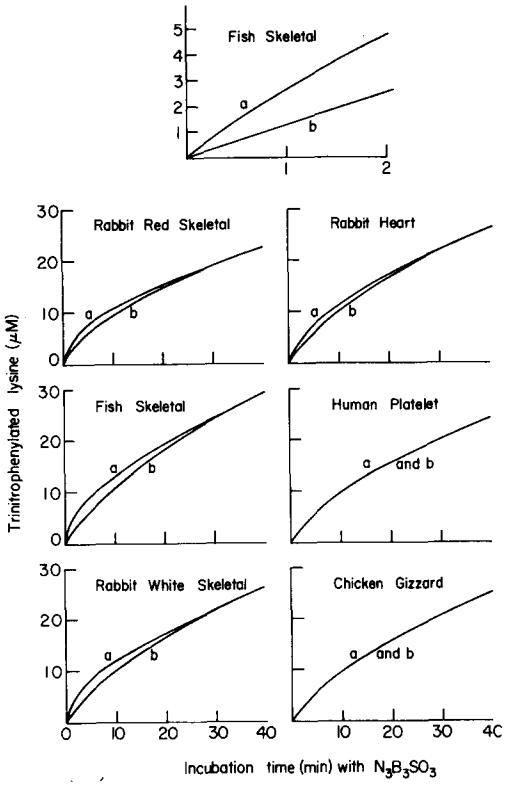


Fig. 1. Kinetics of trinitrophenylation of myosins from different origins. To 1 ml of 1.6 mg/ml (3.2 μM) myosin in 0.6 M KCl and 50 mM Tris/acetate (pH 7.4), 10 μl of 8.3 mM $N_3BzSO_3$ was added (final concentration 83 μM). The reaction was carried out either in the absence (a) or in the presence (b) of 1.5 mM ADP and 2 mM $MgCl_2$. The reaction was followed in a Gilford spectrophotometer at 345 nm using a thermostated optical cell (25°C). The uppermost curves represent the initial change for fish skeletal myosin in extended scales.

independently of the presence of Mg · ADP during incubation with $N_3BzSO_3$. However, large differences were observed for the $v_i$ values, especially if Mg · ADP was absent during the reaction: in the latter case, $v_i$ decreased in the following order: fish skeletal, rabbit white skeletal, rabbit red skeletal, rabbit heart, human platelet and chicken gizzard myosin. As can be seen from Fig. 1, the rate of reaction of the fast reacting amino groups was decreased by the addition of Mg · ADP, except for gizzard and platelet myosins where no Mg · ADP dependence was found.

The effect of trinitrophenylation on the $Mg^{2+}$- and $K^+$-moderated ATPases of myosins was also followed. The time course of the ATPase activities of red skeletal and of gizzard myosins are given in Fig. 2A and the activities of all myosins when two $N_3BzSO_3$ molecules per myosin molecule were incorporated are shown in Fig. 2B. The $K^+$-activated ATPase of heart and of all skeletal muscle myosins strongly diminished and their $Mg^{2+}$-mediated ATPase activity increased and reached a plateau after several minutes if the reaction was carried out in the absence of Mg · ADP. The changes in activities in the presence of Mg · ADP were much more moderate. The activities of smooth muscle and

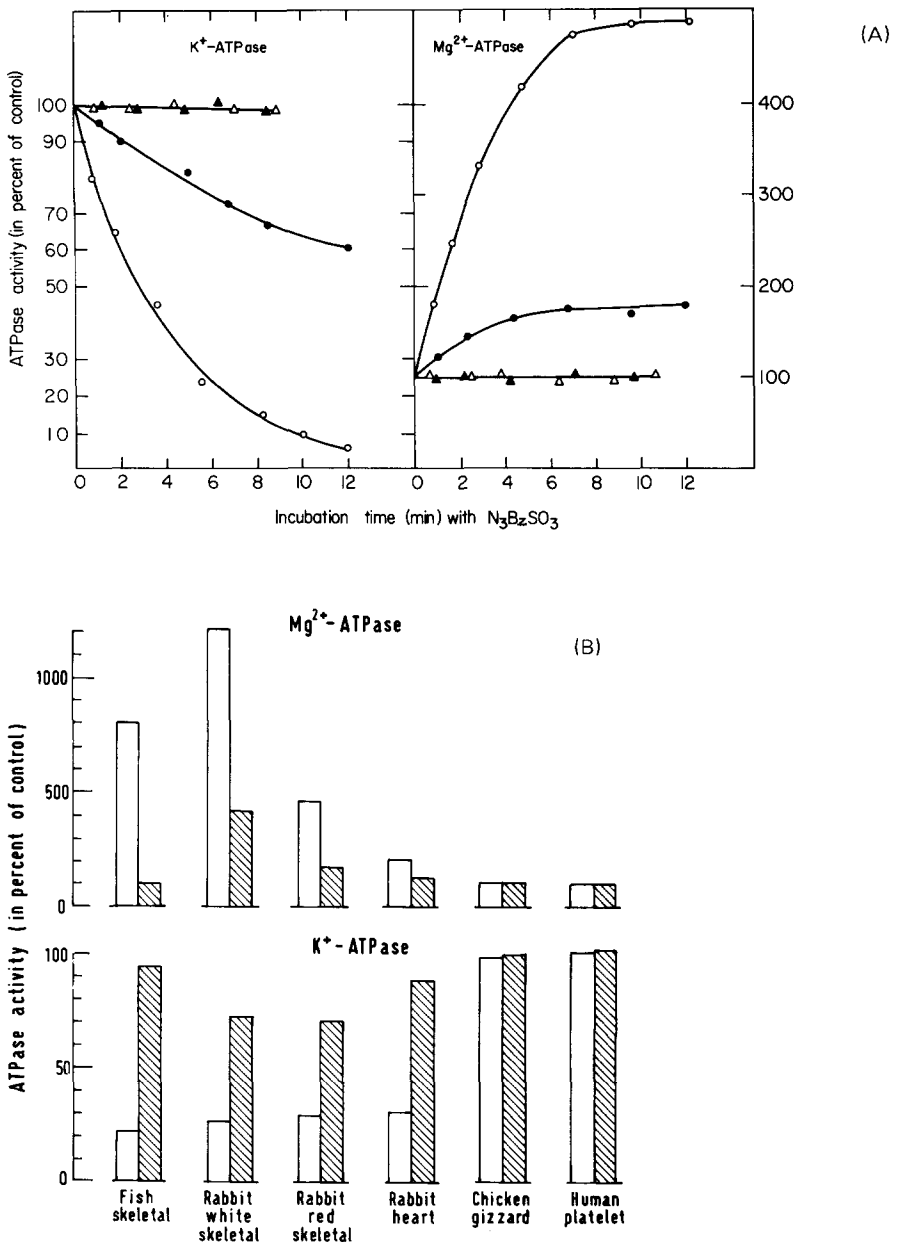


Fig. 2. Effect of trinitrophenylation on the $K^+$-and the $Mg^{2+}$-dependent ATPase activities of myosin. Myosin was allowed to react with $N_3BzSO_3$ under the conditions described for Fig. 1 and aliquots were withdrawn for ATPase assay at time intervals indicated on the abscissa. For details on ATPase determination see Experimental Procedure. A: ATPase activities of rabbit red skeletal and chicken gizzard myosins versus time of incubation with $N_3BzSO_3$. B: ATPase activities of all myosins after two trinitrophenyl groups per myosin molecule have been introduced. Symbols: A, Circles: myosin from rabbit red skeletal muscle; triangles: myosin from chicken gizzard. A and B, Empty symbols: in the absence of Mg · ADP; filled symbols: in the presence of Mg · ADP. Control activities (in $\mu$mol $P_i \cdot mg^{-1} \cdot min^{-1}$) were: Rabbit white skeletal: $K^+$-ATPase 2.06 and $Mg^{2+}$-ATPase 0.005; fish skeletal: $K^+$-ATPase; 0.92 and $Mg^{2+}$-ATPase 0.004. Rabbit red skeletal: $K^+$-ATPase 0.92 and $Mg^{2+}$-ATPase 0.005; rabbit heart: $K^+$-ATPase 0.71 and $Mg^{2+}$-ATPase 0.0055; chicken gizzard: $K^+$-ATPase 0.53 and $Mg^{2+}$-ATPase 0.004; human platelet: $K^+$-ATPase 0.49 and $Mg^{2+}$-ATPase 0.006.

TABLE I

INITIAL VELOCITIES OF TRINITROPHENYLATION OF VARIOUS MYOSINS BY TRINITROBENZENE SULFONATE

Velocities, in μM per min, of ε-amino groups trinitrophenylated by the reagent under the conditions of the reaction (see Experimental Procedure).

| Myosin | Spectrophotometric | | | | | | Enzymic | |
|---|---|---|---|---|---|---|---|---|
| | without Mg · ADP | | | with Mg · ADP | | | without Mg · ADP | with Mg · ADP |
| | $v$ | $v_1$ | $v_2$ | $v$ | $v_1$ | $v_2$ | | |
| Fish skeletal | 3.12 | 2.38 | 0.74 | 1.36 | 0.64 | 0.72 | 2.06 | 0.066 |
| Rabbit white skeletal | 2.97 | 2.35 | 0.62 | 1.37 | 0.76 | 0.61 | 1.98 | 0.41 |
| Rabbit red skeletal | 2.31 | 1.92 | 0.49 | 1.38 | 0.89 | 0.49 | 1.58 | 0.32 |
| Rabbit heart | 1.94 | 1.33 | 0.61 | 1.34 | 0.74 | 0.60 | 1.32 | 0.19 |
| Chicken gizzard | 1.38 | 0.82 | 0.56 | 1.31 | 0.75 | 0.56 | — | — |
| Human platelet | 1.47 | 0.93 | 0.54 | 1.42 | 0.92 | 0.50 | — | — |

platelet myosins were not affected by trinitrophenylation.

In the case of heart and skeletal muscle myosins the rates of trinitrophenylation of the essential amino groups could be evaluated from the plot against time of the logarithm of the $K^+$-ATPase activity which gave a linear dependence, showing that the reaction was first order with respect to the active sites. Considering that the reaction rate was proportional also to the initial $N_3BzSO_3$ concentration [7], the rate constants for different skeletal myosins were calculated and presented in Table II. The rate constants for the reactions carried out in the absence of Mg · ADP were found to decrease in the following order: fish skeletal muscle, rabbit white skeletal muscle, rabbit red skeletal and rabbit heart muscle myosins. Smaller constants were obtained for myosins trinitrophenylated in the presence of Mg · ADP.

It is possible to evaluate the initial velocities in terms of μmoles lysine reacted per min from the change with time of the $K^+$-ATPase if one knows the number of essential lysine residues per mole of myosin. In order to find this value the percentage of remaining $K^+$-ATPase activity after $N_3BzSO_3$ treatment was plotted against the number of trinitrophenyl groups incorporated per myosin molecule (Fig. 3). All skeletal myosins and heart myosin studied gave the same curve. Extrapolation of the initial slope of this curve gave a value of

TABLE II

RATE CONSTANTS OF THE TRINITROPHENYLATION REACTIONS OF VARIOUS MYOSINS

| Myosin | Rate constant $k_1$ ($M^{-1} \cdot min^{-1}$) | | | |
|---|---|---|---|---|
| | Spectrophotometric | | Enzymic | |
| | without Mg · ADP | with Mg · ADP | without Mg · ADP | with Mg · ADP |
| Fish skeletal | 4463 | 1201 | 3880 | 124 |
| Rabbit shite skeletal | 4421 | 1430 | 3720 | 780 |
| Rabbit red skeletal | 3614 | 1675 | 2960 | 600 |
| Rabbit heart | 2500 | 1380 | 2460 | 358 |

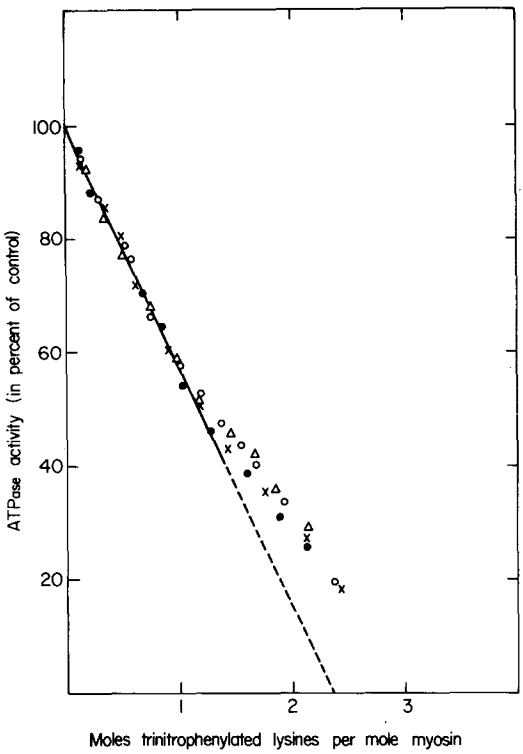


Fig. 3. $K^+$-ATPase of myosin as a function of the total number of trinitrophenyl groups introduced. For details see Figs. 1 and 2. The reaction was carried out in the absence of Mg · ADP. Symbols: •, fish skeletal; ×, rabbit white skeletal; ○, rabbit red skeletal; △, rabbit heart. For control activities see Fig. 2.

2.3 for the number of bound trinitrophenol groups in the fully modified myosin ATPase. This was taken as an indication that there are two essential amino groups per molecule for all myosins the ATPase activity of which has been affected by trinitrophenylation. We attributed the somewhat higher experimental value of 2.3 to the contribution of lysine groups which react with $N_3BzSO_3$ without this affecting the ATPase activity.

The initial velocities of the fast-reacting amino groups were evaluated on the basis of the change with time of the $K^+$-activated ATPase activity and are given on the right-hand side of Table I. If one compares these values with those obtained from the analysis of the spectrophotometric curves ($v_1$), a reasonably good agreement is observed, especially if trinitrophenylation was carried out in the absence of Mg · ADP. However, there were discrepancies between the rate of loss of enzymic activity and $v_1$ if the reaction was carried out in the presence of Mg · ADP, the $v_1$ values being larger. This fact, together with the finding that smooth muscle and platelet myosins also contain amino groups which react fastly with $N_3BzSO_3$ without this affecting the ATPase activity, again indicates that besides the essential amino groups there might be other lysyl residues which are sensitive to $N_3BzSO_3$.

Assuming that there are two fast reacting amino groups in the two active sites of myosin, i.e., one such group per active site, one can calculate the

second-order rate constants ($k_1$) of the reaction of these groups with $N_3BzSO_3$ (see Table II) from the $v_1$ values presented in Table I.

The effect of alkylation of the essential thiol groups on the $K^+$-and $Ca^{2+}$-activated ATPase activity myosins from different sources was also studied. If the MalNEt treatment was carried out at 0° C and in the absence of nucleotide (i.e., conditions under which only the $-SH_1$ thiols of the white skeletal myosin were reported to react [2]), then a step decrease was observed in the $K^+$-activated and an increase in $Ca^{2+}$-moderated ATPase which reached a plateau 5—6 min after the beginning of the reaction in all myosins studied (Fig. 4). However, the maximal activation of $Ca^{2+}$-moderated ATPase depended on the source of myosin. The extent of activation was observed to decrease in the following order: rabbit white skeletal, fish skeletal, rabbit red skeletal, rabbit heart, human platelet and chicken gizzard myosin.

Alkylation was also carried out in the presence of MgADP at 25° C when both the $-SH_1$ and $-SH_2$ thiols of rabbit white skeletal myosin were claimed to react [2]. As a consequence of the MalNEt reaction under the above conditions, the $K^+$-activated ATPase was found to decrease (Fig. 5A) in all myosins; however, differences were found in the rate of decrease, this being smallest for heart and smooth muscle myosins. The $Ca^{2+}$-moderated ATPase activity initially increases for all myosins (Fig. 5B); however, in the later phase of alkylation differences were observed in the behavior of $Ca^{2+}$-ATPase activities of different myosins: the $Ca^{2+}$-ATPase activities of all skeletal myosins and of platelet myosin decreased, while heart and smooth muscle myosins showed a plateau.

Watterson and Schaub [18] suggested to use the rate of alkylation of the

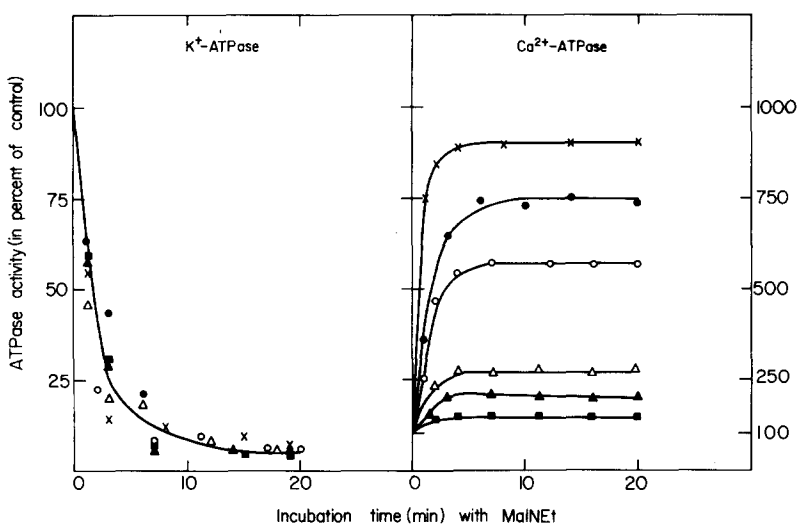


Fig. 4. Effect of alkylation with MalNEt on the $K^+$- and the $Ca^{2+}$-activated ATPase activities of myosin. The reaction was carried out at 0° C in the absence of Mg · ADP, as described in the Experimental Procedure. Myosins: ●, fish skeletal; ×, rabbit white skeletal; ○, rabbit red skeletal; △, rabbit heart; ■, chicken gizzard; ▲, human platelet. Control activities, in $\mu mol\ P_i \cdot mg^{-1} \cdot min^{-1}$: fish skeletal: $K^+$-ATPase 1.20 and $Ca^{2+}$-ATPase 0.096; rabbit white skeletal: $K^+$-ATPase 2.06 and $Ca^{2+}$-ATPase 0.21; rabbit red skeletal: $K^+$-ATPase 0.92 and $Ca^{2+}$-ATPase 0.14; rabbit heart: $K^+$-ATPase 0.71 and $Ca^{2+}$-ATPase 0.16; chicken gizzard: $K^+$-ATPase 0.53 and $Ca^{2+}$-ATPase 0.15; human platelet: $K^+$-ATPase 0.49 and $Ca^{2+}$-ATPase 0.18.

TABLE III

INACTIVATION BY MalNEt OF THE $K^+$-ACTIVATED ATPase ACTIVITY OF VARIOUS MYOSINS

| Myosin | Rate of inactivation per μM MalNEt added | | | | | | Ratio of inactivation rates | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Without nucleotides | | With Mg · ADP | | With Mg · ATP | | Mg · ADP / Mg · ATP | | No nucleotide/Mg · ATP | | No nucleotide/Mg · ADP | |
| | in 60 mM KCl | in 0.6 M KCl | in 60 mM KCl | in 0.6 M KCl | in 60 mM KCl | in 0.6 M KCl | in 60 mM KCl | in 0.6 M KCl | in 60 mM KCl | in 0.6 M KCl | in 60 mM KCl | in 0.6 M KCl |
| Fish skeletal | 2.3 | 1.8 | 1.3 | 1.25 | 0.34 | 0.72 | 3.8 | 1.75 | 6.8 | 2.5 | 1.8 | 1.44 |
| Rabbit whise skeletal | 4.9 | 2.2 | 4.8 | 3.2 | 0.9 | 1.1 | 5.3 | 2.9 | 5.45 | 2.0 | 1.0 | 0.69 |
| Rabbit red skeletal | 4.4 | 1.34 | 3.3 | 2.0 | 1.8 | 1.2 | 1.85 | 1.67 | 2.45 | 2.16 | 1.33 | 1.3 |
| Rabbit heart | 3.1 | 1.34 | 1.5 | 0.50 | 1.2 | 0.10 | 1.25 | 5.0 | 2.58 | 13.4 | 2.06 | 2.68 |
| Chicken gizzard | 3.2 | 2.9 | 1.9 | 2.8 | 1.7 | 2.5 | 1.11 | 1.11 | 1.9 | 1.15 | 1.7 | 1.04 |

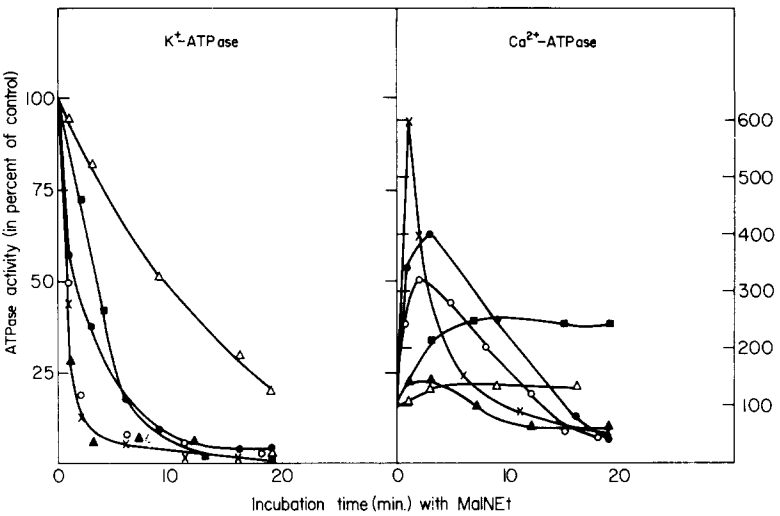


Fig. 5. Effect of alkylation with MalNEt in the presence of Mg · ADP on the $K^+$- and the $Ca^{2+}$-ATPase activities of myosin. The reaction was carried out at 25° C in the presence of 2.5 mM ADP and 5 mM $MgCl_2$ as described in the Experimental Procedure. For symbols and control activities see Fig. 4.

essential -SH groups of myosin as a measure of a conformational state of the protein during different stages of the steady-state ATP hydrolysis. This can be performed by alkylating myosin for increasing periods of time at low protein concentration with subsequent measurement of the $k^+$-activated ATPase. The alkylation was carried out with five different myosins at 25° C at low or high ionic strength, in the presence of either Mg · ADP or Mg · ATP or in the absence of nucleotides (Table III). The difference in the rate of inactivation under different conditions reflects on the alteration of the structural state of the active site from one set of conditions to another. Our first concern was to see the differences which exist in the rate of inactivation in the $K^+$-dependent ATPases of myosin when either Mg · ADP or Mg · ATP was present during alkylation. In the majority of cases, the rate of inactivation was smaller in the presence of Mg · ATP than with Mg · ADP, suggesting differences in the conformation of myosin. The only exception was the smooth muscle myosin at both ionic strengths and the heart myosin at low ionic strength, where differences hardly existed in the rates of inactivation observed in the presence of Mg · ADP and Mg · ATP. The addition of nucleotides generally decreased the inactivation rates except for Mg · ADP in the case of rabbit white skeletal muscle (at low ionic strength) and smooth muscle myosins (at high ionic strength). In the latter case, Mg · ATP did not significantly influence the rate of loss of enzymatic activity. The rate of inactivation was also found to depend on ionic strength (otherwise under identical conditions): in the majority of cases higher rates were found at low ionic strength, with the notable exception of smooth muscle myosin in the presence of nucleotides, where the opposite trend was observed. However, independently of which ionic conditions favored more alkylation, the result clearly showed that the conformation of the active site of myosin depends on the ionic strength of the environment.

## Discussion

On the basis of the reaction with $N_3BzSO_3$ one could classify the $\epsilon$-amino lysine groups of different myosins into three classes. To the first class belong two amino groups which react rapidly with $N_3BzSO_3$, the reaction being highly retarded in the presence of Mg · ADP and Mg · ATP [6,22] and whose modification causes fundamental changes in the enzymic properties of myosin. This class is absent in smooth muscle and platelet myosins as trinitrophenylation of these myosins does not affect their ATPase activity and the presence of Mg · ADP does not influence the rate of reaction with $N_3BzSO_3$. The second class comprises of amino groups which although they react fastly with $N_3BzSO_3$, their trinitrophenylation does not affect the enzymic properties and their rate of reaction does not depend on the presence of Mg · ADP. Thus, in the case of platelet and smooth muscle myosins, spectrophotometric measurements revealed the existence of a fast trinitrophenylation reaction; however almost no change could be detected in the enzymic behavior. This class could be found in essentially all myosins studied. The rest of the $\epsilon$-amino groups of lysine belong to the third class which reacts with $N_3BzSO_3$ with a slow rate and which comprised the great majority of amino groups of all myosins studied. No fundamental differences could be detected between different myosin with respect to this class of amino groups.

Two essential amino groups were observed in all skeletal myosins studied and in heart myosin. However, quantitative differences were found between these myosins with respect to the rate of their reaction with $N_3BzSO_3$ (Table II) and to the effect of their modification on enzymic activity (Fig. 2B). A correlation was found between these two properties, i.e. the higher the rate of trinitrophenylation the greater the change in enzymic activity. The study of these characteristics shows that myosins from different animals (e.g., rabbit white skeletal and fish skeletal) could be more similar to each other than red and white muscle myosins from the same animal. Similar results were observed with respect to the effects of blocking of -SH groups on the $Ca^{2+}$-dependent ATPase (Figs. 4 and 5). In this case also, the highest activation was observed in myosins from rabbit white skeletal and from fish muscles. All this suggests that the function of a muscle rather than the evolutionary distance could be the most important factor which determines the nature of the active sites of myosin.

Contrary to the amino groups, the thiol residues were found to be essential at least for the $K^+$-dependent ATPase of all myosins studied. However, differences were found in the $Ca^{2+}$-dependent ATPase after alkylation of -SH groups of myosins from different sources. It seems that alkylation of -SH affects the $Ca^{2+}$-activated ATPase of smooth and platelet myosins much less than that of myosins from skeletal muscles.

If MalNEt treatment is carried out in the presence of Mg · ADP, the $Ca^{2+}$-ATPase of skeletal and platelet myosins as function of time shows a maximum. The reason for the loss of activity at higher degrees of alkylation is that under these circumstances the $-SH_2$ thiol groups, which is essential for the $Ca^{2+}$-moderated ATPase activity of myosin, is blocked [2,3]. The $-SH_2$ groups becomes accessible to MalNEt because of a Mg · ADP-induced conformational change in the active site. We could not find a decrease in $Ca^{2+}$-ATPase with

smooth and heart myosins which behave in the same manner whether or not Mg · ADP is present during alkylation (see Figs. 4 and 5). One may therefore assume that Mg · ADP does not induce the structural change which makes the thiols essential for $Ca^{2+}$-ATPase accessible for the reaction or, alternatively, that these thiols do not exist in smooth muscle and heart myosins. Our finding, with respect to the cardiac myosin, is not in agreement with Pfisten et al. [1] who claimed the existence of two thiols essential for the $Ca^{2+}$-ATPase.

In conclusion, we suggest to use chemical modifications as a tool for the characterization of differences in the active sites of different myosins.

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