The action of pyrophosphate on adenosinetriphosphatase activity of myosin*

Recent investigations on myosin adenosine triphosphatase (ATPase) have shown that pronounced enzymic activation can be accomplished by ethylenediaminetetraacetic acid (EDTA) in the presence of ammonium ion or potassium ion[1,2,3]. The present communication reports the activation of myosin ATPase by inorganic sodium pyrophosphate (P-P) in the presence of ammonium or potassium ion, and the effect of P-P on activation by EDTA-ammonium.

The myosin was prepared from rabbit skeletal muscle by a method similar to that of SZENT-GYÖRGYI[4] as modified by PERRY[5]. Although slight myokinase activity and adenylic deaminase activities were present, both enzymic activities were negligible under the conditions described below, notably the lack of magnesium and the relatively high pH.

The amount of phosphate liberated by the action of myosin on ATP was measured by the method of FISKE AND SUBBAROW[6]. It was found essential to remove the P-P after stopping the reaction, before the analysis for orthophosphate, because concentrations of P-P greater than 0.01 M interfere with the determination of orthophosphate. The P-P was removed as follows: To 2 ml of the reaction mixtures described below, 1 ml 15% trichloroacetic acid (TCA) was added, followed immediately by 1 ml 0.3 M $MnSO_4$ in 1 M sodium acetate, resulting in a final pH between 3 and 4. Under these conditions[7], most of the P-P precipitated as the manganous salt, leaving inorganic orthophosphate, ADP and ATP in solution. The solutions were filtered and 1 ml of filtrate was used for orthophosphate determination.

The effect of P-P as an activator of myosin ATPase was measured as follows: To 1 ml of Tris buffer, pH 9.1, containing NH_4Cl and varying amounts of P-P, 0.3 ml of ATP was added; the volume was brought to 1.8 ml with water, and 0.2 ml of myosin in 0.5 M KCl was added; the final pH was 9.1. The final concentrations in 2 ml of reaction mixture were: ATP (0.01 M), NH_4Cl (0.25 M), Tris (0.05 M), and myosin ranged from 25–80 μg/ml. The reactions were run at 25° C for 15 min and stopped with TCA as described above.

To show the effect of P-P on the EDTA-ammonium system, the incubation mixture used was the same as that described above except that EDTA was incorporated to a final concentration of 0.001 M.

In Fig. 1 the lower curve (○) illustrates the P-P–NH_4^+ system without EDTA. This system shows optimum activity at about 0.025 to 0.030 M P-P, approximately 30% of that obtainable using EDTA (0.001–0.05 M) and 0.25 M NH_4^+ without P-P. The P-P–NH_4^+ activation obtained was greater than that found using 0.01 M $CaCl_2$ at pH 9.1 in the usual myosin ATPase assay. It should be mentioned that P-P without NH_4^+ present does not activate the enzyme, and this is analogous to the EDTA system[3]. K^+, replacing NH_4^+, showed similar but lesser activation, and Na^+ had no effect, which is also true for the EDTA system[3]. In the report of KIELLEY, KALCKAR AND BRADLEY[3], 0.25 M NH_4^+ was described as the concentration necessary for optimal activity. Our own experiments confirm these results. However, with 0.025 M P-P in the absence of EDTA, no optimum was found using varying NH_4^+ concentrations up to as high as 0.5 M;

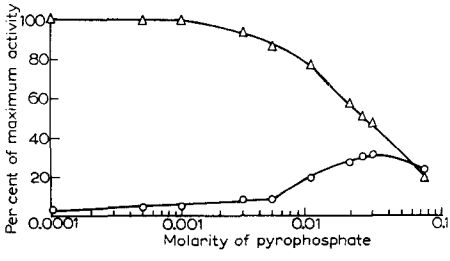

Fig. 1. The effect of increasing the inorganic pyrophosphate concentration is shown in the system containing no EDTA (○) and the system containing 0.001 M EDTA (△). Full activity (100%) is equivalent to the liberation of 5.2 μmoles inorganic orthophosphate.

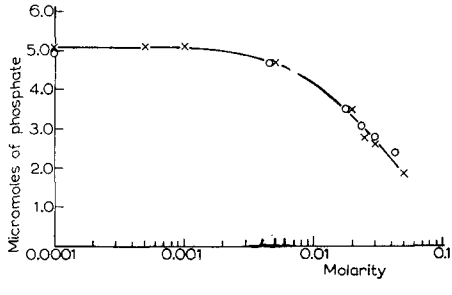

Fig. 2. A comparison is shown of the effects on ATPase activity upon adding inorganic pyrophosphate (×) or additional ATP (○) to the optimal system containing 0.001 M EDTA, 0.008 M ATP, 0.25 M NH_4Cl. The system contained 60 μg myosin/ml. The abscissa (logarithm scale) represents the molarity of added pyrophosphate or of additional ATP.

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the activity continued to rise linearly. In addition, ADP could not replace P-P as an activator, nor could ATP act as its own activator.

The upper curve (△) of Fig. 1 illustrates the results of adding P-P to the EDTA-NH_4^+ system: P-P did not increase the activation, but at higher concentration prevented maximal activation. The two curves approach each other at approximately 0.04 *M* P-P.

In the EDTA-NH_4^+ system containing no P-P, the maximum velocity was obtained with 0.008 *M* ATP. Fig. 2 shows the effect of ATP or inorganic pyrophosphate added to the optimal system. It is clearly seen that the substrate inhibition arising from over optimal amounts of ATP is exactly the same as that obtained by addition of equivalent quantities of P-P. ADP did not show this effect. Inhibition by ATP was not detectable in the P-P-NH_4Cl system lacking EDTA.

No simple explanation of the effects recorded above can be offered. At present, the effects of other triphosphorylated nucleotides and other chelating agents are being investigated; and all of the agents are being further studied using H-meromyosin as the enzyme.

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Esterification of protein and amino acid carboxyl groups by mustard gas and related compounds

From chemical considerations mustard gas and related sulphur compounds (S-mustards) can react with any of the polar groups occurring in proteins and amino acids provided these groups are present in the necessary ionic state. At physiological pH values the carboxyl groups of proteins and amino acids would be in the ionised state and thus would be able to combine with these compounds. Although esterification of the anions of various organic acids by S-mustards and some of the related nitrogen compounds (N-mustards) has been conclusively demonstrated[1,2], evidence for this reaction with protein carboxyl groups is indirect in nature, being obtained either from changes in the titration curves of proteins following treatment with S-mustards[3,4] or from examination of the pH stability of bonds formed between S-mustards and proteins[5,6].

The nucleophilic reagent, hydroxylamine, reacts very readily with esters to liberate an alcohol molecule and form a hydroxamic acid which can be determined by the coloured complex it forms with Fe^{+++} ions. The possibility of using this reagent to follow O-alkylation reactions of S-mustards with protein and amino acid carboxyl groups has therefore been investigated.

Hide powder collagen was treated with radioactive (^{35}S) di-(2-chloroethyl) sulphide (H) essentially as described by PIRIE[5]. The treated collagen was exhaustively Soxhlet-extracted with acetone and ether to remove any unbound radioactive material. Samples of the treated collagen were then incubated for 24 h at pH 7.5 and 38° in the presence of 1.2 *M* hydroxylamine. This treatment resulted in the loss of 56% of the bound H as determined by radioactivity measurements whereas incubation under the same conditions but in the absence of hydroxylamine resulted in the liberation of only 3% of the bound H. When incubation was carried out at pH 11.9, however, 60% of the bound H was liberated in the absence of hydroxylamine. There is thus close agreement between the bound H which is alkali-labile and that which can be liberated by hydroxylamine treatment, as would be expected if hydroxylamine were attacking ester-type linkages between H and collagen.

A further series of experiments was carried out using glycine and several S-mustards. 2 mmoles mustard were allowed to react with 240 mmoles glycine at room temperature in 200 ml water. The pH was maintained at 6.0 to avoid the possibility of any N-alkylation reactions. The large excess of glycine was employed in an attempt to eliminate loss of the reagents by