Reaction of Adenyltriphosphate with Myosin A.

by

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It has been described by BANGA and SZENT-GYÖRGYI that the viscosity[1] of a 24 h. extract of muscle, containing myosin chiefly in the B form, decreases very strongly on addition of adenyltriphosphate (ATP). Under similar conditions the viscosity of a 20 min. extract, containing mainly the A form, decreases but slightly, the cange being only about 10% of the change observed in the 24 h. extract. It has also been shown that this slight decrease of viscosity is not due to myosin A, but to the small quantitities of myosin B present as an impurity.

These experiments have been done with extracts of muscle. Edsall's fluid was used as solvent which is an alkaline 0,6 mol. KCl solution. If the 24 h. extract, which I will call for the sake of convenience „myosin B", is tested in a solution of lower pH and KCl concentration, the viscosity will show an even more pronounced decrease (40% more) on addition of ATP.

Myosin A behaves in an interesting way if the pH and salt concentration are varied. If e. g. myosin A is dissolved in a veronal-acetate buffer of pH 5,3 and the potassium ion concentration is varied by adding different amounts of KCl, ATP will cause a great decrease of viscosity, the magnitude of this effect varying with the salt concentration. Such results are shown in Fig. 1. The maximal value of this ATP effect is quantitatively the same as that which would be obtained with a myosin B solution having the same viscosity. The same

[1] The term viscosity in used in this paper knowing that, in the type of viscometer used in these experiments, deviations from POISEUILLE's law are perceptible. The effects described here, however, are not materially influenced by possible corrections in the viscosity values.

type of curve is obtained if the potassium ion concentration is kept constant and the pH varied.

While myosin A gives a clear solution in EDSALL's alkaline KCl, in solvents in which the ATP effect in increased,

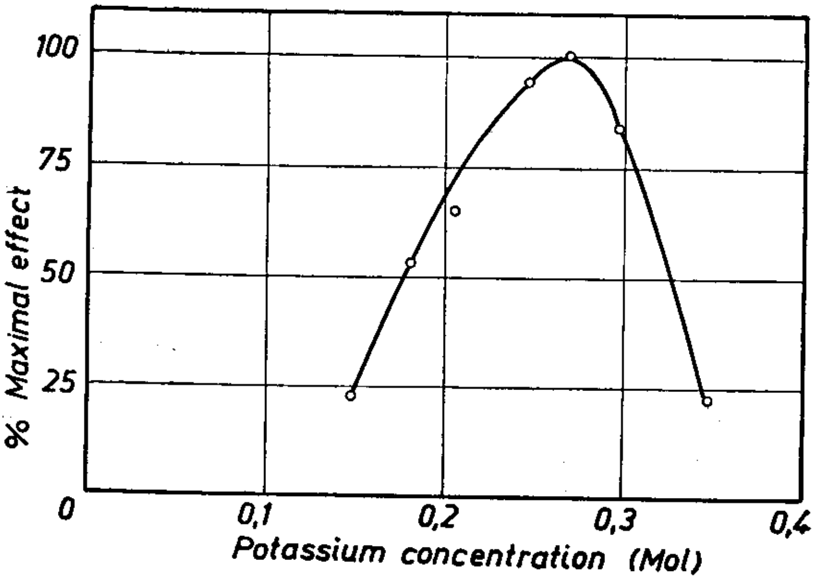

Fig. 1.

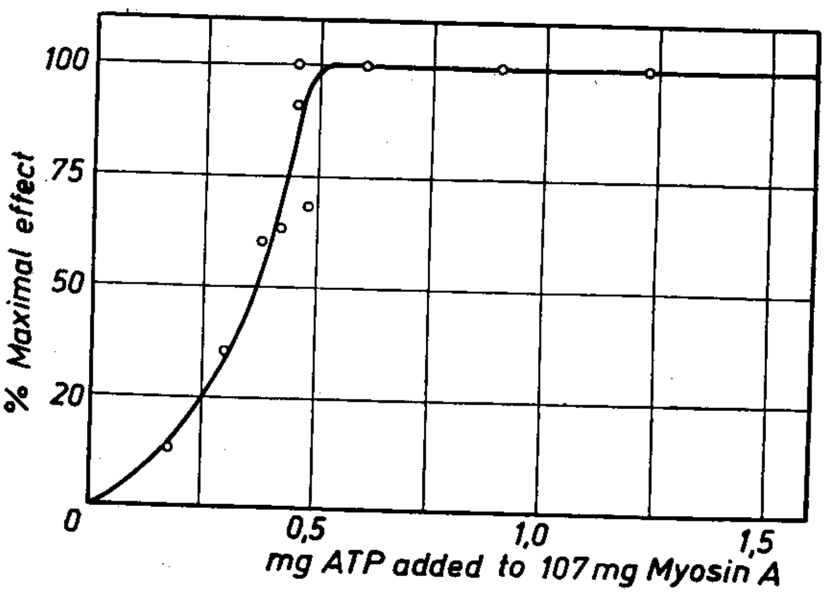

Fig. 2.

the opalescence increases roughly in proportion. Finally, decreasing the potassium ion concentration below its optimum (which gave maximal ATP effect) will result in inreasing flockulation. Considring the combined action of pH and salt concentration, one is led to suppose. that the ATP effect is the function of a particular colloidal state and not of the difference between the two myosin.

It was interesting to see what concentration of ATP is required to give a maximal effect (maximal decrease of viscosity) in these experiments with myosin A. It will be seen from Fig. 2. that 0,5 mg ATP in 30 ml are needed to give a maximal decrease of viscosity with 107 mg myosin A. The combination between ATP and myosin A is therefore just as strong as the combination between ATP and B. Moreover it should be pointed out that in spite of a difference in solubility and viscosity, suggesting a difference in size of the myosin A and B particles, the equivalent reacting weight of myosin with ATP is the same in both cases.

Experiments.

In the experiments recorded in Fig. 1., 5 ml of myosin A solution (100 mg myosin in 0,6 mol. KCl) were added to 25 ml of a buffer solution and the viscosity determined in a capillary vicometer at O°C. The buffer solution consisted of 2,5 ml of 0,28 mol. veronal-K, 2,5 ml of potassium acetate of the same concentration, 8,0 ml n/10 HCl, varying amounts of KCl and distilled water to make up 25 ml. If no KCl was added, the potassium concentration in the final volume of 30 ml was 0,147 mol, resulting from the potassium of the buffer and myosin solutions.

A similar mixture was used in the experiment from which Fig. 2. was constructed. The 30 ml of mixture, analysed in the viscometer, contained the same buffer and 5 ml of a myosin A solution (107 mg myosin), together with so much KCl, as to bring the concentration of potassium ions to 0,25 mol. When the amount of ATP was too small, successive readings in the viscometer did not agree but showed an ever decreasing ATP effect. This is due to the splitting of ATP by myosin. In these cases the viscosity at zero time (at the moment of adding the ATP) was evaluated by linear extrapolation from the values of 3—4 successive determinations. The errors inherent in this extrapolation may account for the fact that the rising part of the curve is not linear, as could be expected.

Summary.

It is shown that, at a lower pH and a lower salt concentration, myosin A also has a high viscosity which is decreased by ATP. ATP reacts with myosin A in the same proportion as with myosin B (one g. mol. ATP per 100,000 g of myosin).