Quantitative Studies on some Effects of Adenyltriphosphate on Myosin B.

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The question discussed in this paper is the following: which is the minimal quality of adenyltriposphate („ATP") which still gives rise to a maximal effect on myosin?

The effects of ATP on viscosity, on light scattering and on precipitation have been studied.

1. The viscosity-lowering effect. The relative viscosity of a solution of myosin B is high; addition of ATP causes a marked fall. It is, however, not possible to determine the quantitative relations between ATP and myosin simply from this, because the viscosity-lowering effect of very small quantities of ATP decreases with time, apparently due to the splitting of ATP by myosin. The influence of time has therefore to be taken into account.

0,2—0,3% solution of myosin in Edsall's fluid were used and measurements taken with a viscosimeter of the Ostwald type, which was immersed in ice-water.

Results are given as specific viscosity; the differences in density of the solution and the solvent have not been taken into account in the calculations, this correction being of no importance, as all determinations of one series of experiments were made with solutions of the same concentration.

First the viscosity of the myosin solution was determined. The fluid was then poured out of the viscosimeter into a tube, likewise suspended in ice-water and the viscosimeter immediately put back in its place. After a short time dissolved ATP was added in an amount which did not cause dilution of the myosin solution by more than 0.1%. The time of complete mixing was noted. The solution was then put back

into the viscosimeter, with a pipette surrounded by a mantle of ice-water. If care is taken to avoid warming of the solution or instruments, the first reading can be made immediately, without error due to a rise in temperature. Measurements were repeated every 3 or 4 minutes, usually for 20 to 40 minutes.

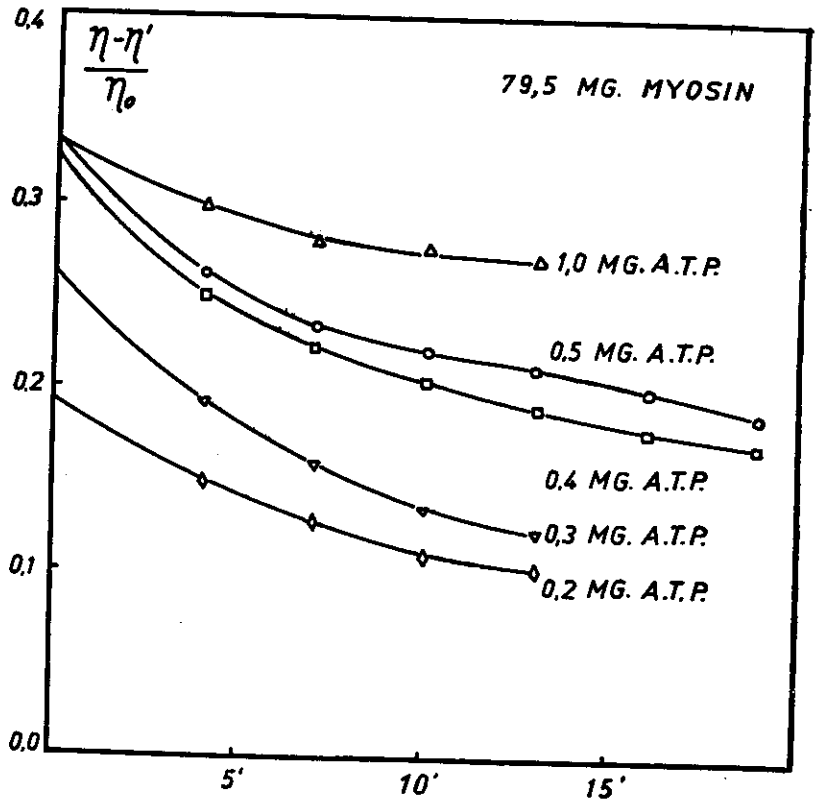

Fig. 1.

The graphs show the viscosity-lowering effect as the difference between the specific or relative viscosities of the solution with and without ATP at different moments after mixing.

An example of a series of experiments is shown in fig. I. As will be seen, the viscosity-lowering effect decreases regulary with time, an end-value usually being reached within 15 to 45 minutes. Extrapolation of the curves towards

zero time gives the magnitude of the initial effects. The figures in this example show that 1,0 and 0,5 mg ATP had the same effect; 0,4 mg acts slightly less and still smaller doses of ATP are clearly submaximal.

In Fig. 2 the results of a number of series of experiments have been plotted in the following way: the ordinate shows the effect of each ATP quantity as the percentage of the maximal effect in the same series; the abscissa gives the ATP quantity present per 100 mg of myosin.

In the range of lower concentrations the spreading of the

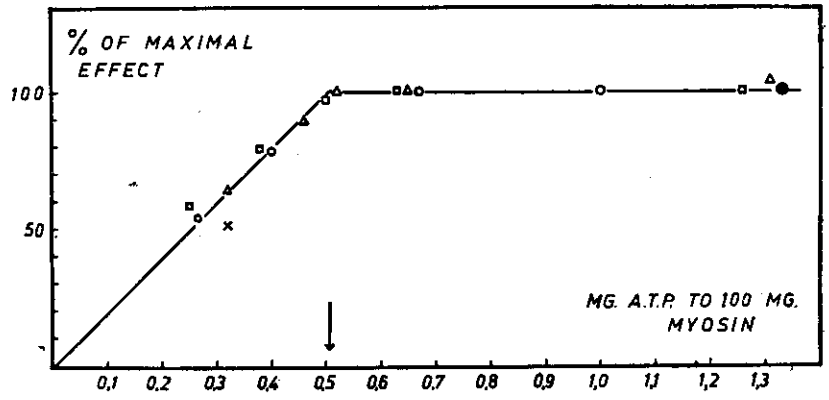

Fig. 2.

points is considerable owing to the fact that in the case of actions far below the maximum the extrapolation of the time-curves is difficult. In the neighbourhood of the maximal effect it can be made with sufficient accuracy.

The relation between the ATP quantity and the magnitude of the effect in the submaximal range seems to be a linear one: the transition towards the horizontal part of the curve is very sharp; the transition-point, which indicates the minimal fully effective ATP quantity, seems to lie between 0,50 and 0,51 mg ATP per 100 g myosin. From this the weight of the reacting myosin-unit may be calculated to be about 100,000.

2. The effect on light-scattering. As has been decribed in another paper of this series (BANGA and SZ.) the turbidity of myosin B solutions is higher than the turbidity of solutions of myosin A. Addition of ATP in sufficient

quantity to the former causes a decrease of turbidity by 20—30%.

Measurements were made with the Zeiss Stupho-nephelometer using the S. 53 greenfilter. The lightscattering of different samples of myosin solution was measured, before and after the addition of a known amount of ATP. Like in the case of viscosity, the ATP-effect depends on time and the real value can be found by the extrapolation of the time-curve towards zero; however, the mean value of 5 readings made

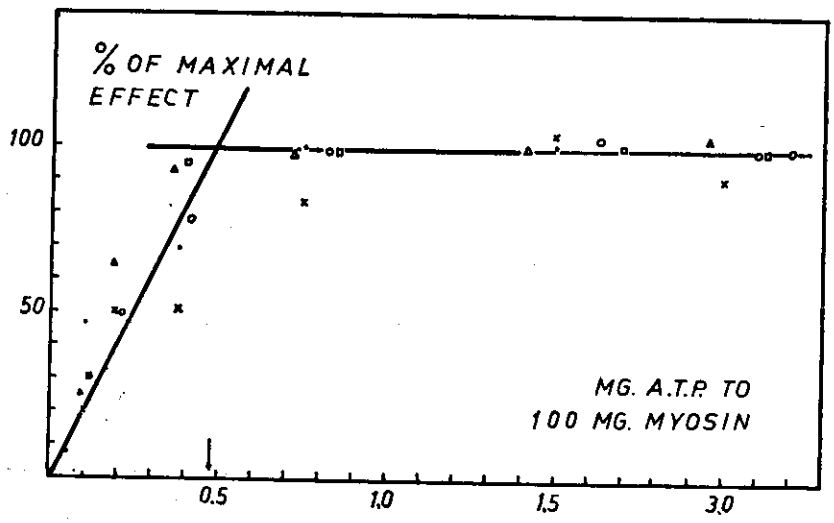

Fig. 3.

within 1,5 minutes after the addition of ATP, can be used as a base of comparison just as well as the extrapolated value. All measurements were made at room temperature (30°C.)

The results of a number of series of experiments, made with different preparations containing 0,4—2,0 mg myosin per ml are given in fig. 3. The spreading of points is considerable owing to the smallness of the effects; it is even difficult to decide about the exact form and situation of the curve. The line which has been chosen as the most probable one points to 0,47 mg ATP per 100 mg of myosin as the minimal quantity giving full effect; this value is almost the same as that got with the viscosimentric method (0,51).

It may, therefore, be concluded that the study of the

effect of ATP on light-scattering in myosin solutions is not in disagreement with the results of the viscosity measurements.

3. The effect on gel-volume. If a myosin preparation, dissolved in Edsall's fluid, is diluted with a five-fold volume of distilled water and neutralised with acetic acid, a precipitate is formed. The precipitation becomes more intense on addition of ATP and correspondingly the volume of the precipitate becomes smaller.

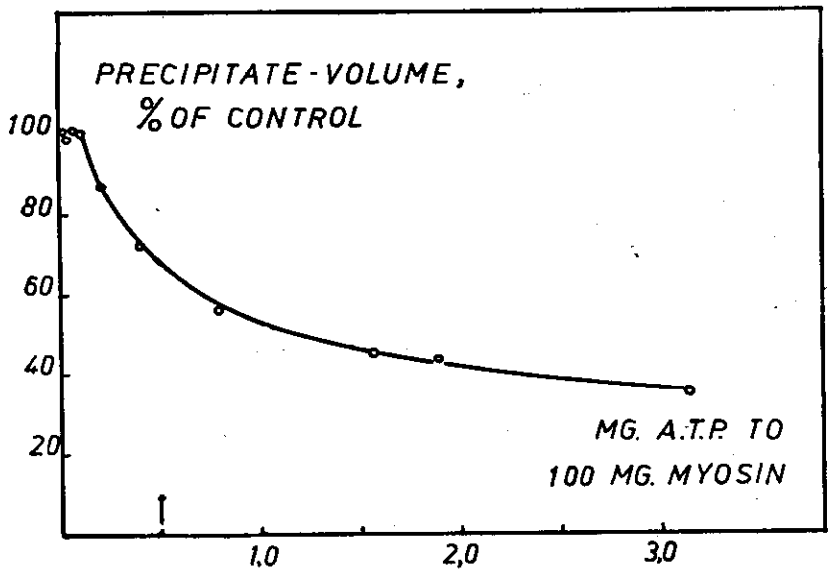

Fig. 4.

For the determination of the volume of the precipitate, 1 ml of myosin solution (in most cases containing 12 mg per ml) was mixed with 5 ml of distilled water and different amounts of ATP in a weighed cetrifuge tube of 10 ml. The volume of the precipitate was determined by weighing it in the wet state, the tube having been dried carefully with filter paper. Neutralisation was found not to be essential for precipitation, although the gel settled more easily and with a smaller volume after neutralisation; this is in agreement with the results of Edsall (1). Common distilled water had to be used because no well-formed gel could be obtained in glass-distilled water. The most regular results were obtained by precipitating the myosin first and then adding the ATP; similar results, only

with somewhat scattered data, were obtained if the myosin solution was diluted with water already containing ATP. If first the ATP was added to the myosin and dilution carried out after the elapse of a few seconds or minutes, higher ATP concentrations were needed in order to get the same effect, which was doubtless due to the splitting of the ATP in the concentrated myosin solution. This method seemed, therefore, to be less suited for the present purposes.

Fig. 4 shows the mean values of the results of six series of experiments. As will be seen, a clear maximal effect was not reached, not even in the case of the highest ATP doses. For the explanation of this fact it should be mentioned that between the addition of ATP and the determination of the gel-volume a time of 20 to 25 minutes elapses. Therefore the ATP added will in greater part be split during this time, thus making addition of a larger amount necessary and smoothening the sharp curve. It will be seen, however that at 0,5 mg ATP per 100 mg of myosin the effect is considerable and differs from that of the higher doses by an amount which can be expected to be caused by the splitting of ATP during the experiment. It can be concluded therefore, that the results of these experiments agree in order of magnitude with the results obtained by other methods (The arrow marks the value corresponding to 1 mol. ATP per 100,000 g myosin).

4. Conclusion. From the quantitative study of the effects of ATP on the physical properties of myosin one can conclude that myosin reacts in units of 100.000, a number which seems to be related to the molecular weight in urea solution as found by WEBER and STÖVER (*2*). Every single unit reacts with one molecule of ATP. This result is based on three entirely different methods of investigation in which myosin was studied at O°C and at room temperature, in solution and as a precipitate, in the streaming as well as the resting condition. From the sharpness of the transition point of curve *2* it must be concluded that the myosin-ATP-compound is, at least at O°C, not dissociated to a marked degree.

Literature.

1. *J. T. Edsall:* Jl. of Biol. Chem. *89*, 289, 1930.
2. *H. H. Weber* and *R. Stöver:* Biochem. Z. *259*, 269, 1933.