

A New Protein Factor Promoting Aggregation of Tropomyosin

It has been shown (1, 2) that the physiologically important nature of the contractile system responsive to the regulating action of calcium ions (3, 4) is due to the presence of a protein other than actin and myosin in the system. The protein qualitatively resembles tropomyosin and is called 'tropomyosin-like protein', but it has a larger sedimentation constant and a higher viscosity than those of the Bailey-type tropomyosin (5).

When the 'tropomyosin-like protein' in *M* KCl was brought to pH 4.6, most of the protein was precipitated, whereas a smaller but a significant part, from one-eighth to one-fourth, remained in the supernatant. The precipitate retained most of the properties of the 'tropomyosin-like protein', but its viscosity was lowered to the same level as that of ordinary tropomyosin. If the supernatant, which itself showed low viscosity, was added to the solution of the precipitate, the previous viscosity was restored. This effect of the supernatant was essentially the same on ordinary tropomyosin.

Effective principle in the supernatant was isolated and was shown to have a single peak in the ultracentrifugation diagram, its $s_{20,w}$ being 3.0 S. In contrast to tropomyosin, its intrinsic viscosity was very low, *i.e.*, 0.12 dl./g. even at low ionic strength (~ 0.001). Fig. 1 shows the relative viscosity of ordinary tropomyosin and of the precipitate mentioned above, plotted against increasing concentrations of the isolated factor. These results together with those from the flow birefringence studies clearly indicate that the factor promotes the association of tropomyosin. The factor, however, did not exert any effect on myosin, and if a pure preparation of actin was used, it showed practically no influence on the behavior of actin.

Thus, the 'tropomyosin-like protein' is now divided into two different components. The unique physiological function of the

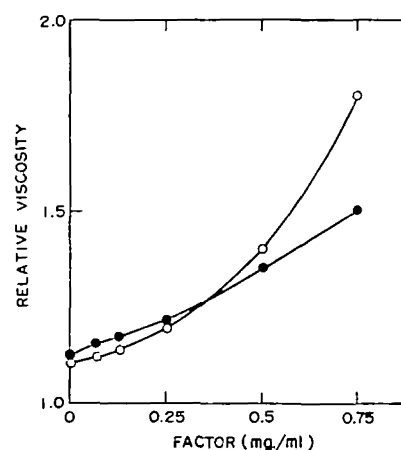


FIG. 1. Effect of aggregation-promoting factor on the viscosity of tropomyosin.

Abscissa indicates the concentration of aggregation-promoting factor (its reduced viscosity at 1 mg./ml. was 0.14 dl./g. under the experimental conditions) added to the solution of tropomyosin of Bailey-type (○) or that of the precipitate from 'tropomyosin-like protein' in *M* KCl at pH 4.6 (●). Final concentrations: 1 mg./ml. tropomyosin or the 'precipitate' 0.08 *M* KCl and 0.02 *M* Tris-maleate (pH 6.8). Temperature, 20°C. Velocity gradient of Ostwald-type viscometer used, 3,200 sec.⁻¹.

'tropomyosin-like protein' mentioned above is retained by the precipitate to some extent, whereas the aggregation-promoting factor isolated from the supernatant does not show any of this action, if experiments are carried out with synthetic actomyosin. In the case of trypsin [EC 3.4.4.4]-treated 'natural' actomyosin (1, 2), however, the factor can exert an effect similar to that of the 'tropomyosin-like protein'. At present no plausible explanation can be given to this fact.

As is well known, tropomyosin exhibits an anomalous viscosity, *e.g.*, its reduced viscosity increases remarkably with the elevation of its concentration. This tendency is further accelerated by the present factor.

This suggests that tropomyosin complex, consisting of tropomyosin and the aggregation-promoting factor, may greatly contribute to viscoelastic properties of the contractile system in living muscle.

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