Studies on Chicken Actomyosin

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There is evidence that the physical properties of actomyosin may vary depending on the physiological state of the muscle from which it was isolated, although the factors responsible for such variation have not been fully elucidated. A comparative study has been made of certain properties of chicken breast actomyosin isolated from pre-rigor (fresh) muscle and muscle taken from whole birds that have been held (aged) at 25°C after death.

The activity of the Mg^{2+} -stimulated adenosine triphosphatase of 'fresh' actomyosin decreased as the ionic strength of the test solution was increased, whereas the actomyosin Mg^{2+} -stimulated adenosine triphosphatase obtained from muscle 'aged' for 2-4 h at 25°C was less sensitive to inhibition by potassium chloride. If the inhibition by potassium chloride of Mg^{2+} -stimulated adenosine triphosphatase activity of rabbit actomyosin was due to the displacement of Mg^{2+} by K^+ from binding sites on the actin-myosin complex, as suggested by Nihei, Morris & Jacobsen (1966), then the observations made in the present study might suggest that Mg^{2+} was not so readily lost in the case of actomyosin from 'aged' chicken muscle.

Superprecipitation (measured by the method of Ebashi, 1961) of actomyosin from 'aged' muscle was rapid and only occasionally was a clear short phase observed in the presence of 75 mM-potassium chloride, whereas actomyosin from 'fresh' muscle was superprecipitated at a much lower rate and exhibited a clearing phase in the presence of 50 mM- and 75 mM-potassium chloride.

It was found that the rate of superprecipitation of actomyosin from 'fresh' muscle was greater at 10 mM- Mg^{2+} than at 2.5 mM- Mg^{2+} , whereas in the case of muscle 'aged' at 25°C for 2 h the rate of superprecipitation of the actomyosin was greater at 2.5 mM- Mg^{2+} than at 10 mM- Mg^{2+} .

The acceleration of superprecipitation of actomyosin from 'aged' muscle suggests that the actin-myosin interaction may have been strengthened or that the content of a regulatory protein has been altered in these preparations. Further, changes of this kind might also be responsible for the observed decreased inhibitory effect of potassium chloride on the Mg^{2+} -stimulated adenosine triphosphatase activity of actomyosin from 'aged' chicken breast muscle.

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An Improved Method for the Electrophoresis of Enzymes using a Polyacrylamide-Starch Gel

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A thin (1 mm-thick) polyacrylamide-starch two-phase gel produces better resolved bands than starch or polyacrylamide alone.

A suitably buffered 7% polyacrylamide gel is prepared in a glass mould measuring 22 cm × 15 cm × 1 mm deep, the air being excluded with a glass plate during gelling. A strip measuring 12 cm × 0.5 cm midway between the anode and cathode is removed by cutting around a glass strip of the same dimensions. The resulting cavity across the gel is carefully filled with 7% buffered starch gel. The samples are introduced into the starch phase, soaked on pieces of cotton thread or Whatman filter paper 1 cm long. Horizontal unidimensional electrophoresis is carried out under the appropriate conditions, with a cooling plate.

Other large-pore gels were tried in place of starch, but the results proved to be inferior. The running of the enzyme from the large-pore starch gel into the smaller-pore polyacrylamide gel results in the bands of activity concentrating at the interphase and running into the polyacrylamide as sharply resolved bands.

This technique has proved particularly effective in the study of the C_5^+ phenotype of pseudocholinesterase (Harris, Hopkinson, Robson & Whittaker, 1963; Robson & Harris, 1966). The serum samples were diluted 1:1 with gel buffer (0.125 M-succinic acid-0.147 M-tris, pH 4.8) and run overnight (16 h) at 7 V/cm with a tank buffer of 0.05 M-citric acid adjusted to pH 4.8 with 40% sodium hydroxide. The zones of pseudocholinesterase activity were localized about 2 cm towards the anode by means of an agar overlay containing the reaction mixture (Stern & Lewis, 1962).

After storage of the serum samples at -20°C for a few weeks, slow-running 'storage' bands developed behind the C_4 band in the C_5^- type and behind the C_5^+ band in the C_5^+ type. These were removed by adding 2-mercaptoethanol to the diluted serum samples to a final concentration of 1 part in 500.

Under these conditions polymorphic variants of adenylate kinase can also be clearly detected (Fildes & Harris, 1966) on the same plate, but in a position cathodic of the origin.

This technique is also being successfully used on dried bloodstains at this laboratory.

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