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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Factors Affecting the Interactions of Proteins Isolated from Erythrocyte Membranes

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Proteins may be isolated from membranes either by a series of extractions with a sequence of reagents of increasing severity or by a one-step procedure aimed at releasing all the protein into solution by exposure of the membrane to just one solvent. Both approaches have their merits, the advantage of the latter being that it produces a solution containing all the membrane proteins without their having been exposed to a range of extractive procedures, its major disadvantage being the very complexity of the solution it produces.

The butanol procedure one of us devised some time ago (Maddy, 1966) had the merit of producing virtually all the protein of erythrocyte 'ghosts' in an aqueous solution in a relatively undamaged state, but unfortunately this protein solution proved to be extremely difficult to fractionate. However, if the butanol procedure is carried out at low ionic strength in the presence of EDTA the resulting protein solution can be readily fractionated. Using polyacrylamide-gel electrophoresis and analytical centrifugation we have demonstrated in this solution (i) a fraction identical with that part of the membrane's protein that may be extracted from the 'ghost' by EDTA solution alone (Marchesi, Steers, Marchesi & Tillack, 1970), (ii) a fraction containing most and probably all of the membrane's sialoprotein, and (iii) a component with a pronounced tendency to aggregate into an intractable complex.

Three sets of interactions have been detected as factors responsible for aggregation of the isolated membrane proteins: a hydrophobic force, identified by the effects of increasing the ionic strength of the solution, a bivalent-ion bridge effect reversed by EDTA or low pH, and an electrostatic interaction between sialoproteins and other protein of opposite charge. Other interactions not destroyed by sodium dodecyl sulphate or guanidine hydrochloride have been observed, but their physical basis is unknown. The influence of these forces on the behaviour of ox erythrocyte membrane proteins after an EDTA-butanol isolation will be considered. The relative effects of the interactions will differ in different methods of protein isolation, but whatever method is employed their existence must be recognized and accounted for.

Recombination *in vitro* of Proteins and Lipids from Mammalian Erythrocyte Membranes

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Membrane apoproteins of mammalian erythrocytes, isolated by the butanol fractionation procedure of Maddy (1966), have been used in recombination experiments carried out in aqueous media with both erythrocyte total lipids and phospholipids. By using density-gradient centrifugation it was found that no recombination occurred at physiological pH, probably owing to repulsion of both negative net charges of proteins and lipids. However, recombinations turned out to be possible with negatively charged sonicated lipids and positively charged proteins between pH 3.5 and 4.5 (Zwaal & van Deenen, 1970). For this purpose the sialic acid was removed from either the proteins or the intact erythrocytes by treatment with neuraminidase before the recombination procedure. The stoichiometry of the reaction appeared to be dependent on pH. The differences in lipid uptake as a function of pH for apoproteins were compared with the differences in uptake of additional lipids by sonicated 'ghost' suspensions and by lipoproteins obtained after pentanol solubilization of erythrocyte membranes (Zwaal & van Deenen, 1968). It appeared that between pH 3.5 and 4 the amount of lipid taken up by the apoproteins was identical with the amount taken up by the 'ghost' suspensions (or lipoproteins) plus the amount that was already present. The measurements on 'ghosts' suggest that at pH 4 no combination with excess of lipids may occur, whereas the apoprotein at this pH are still able to recombine up to a phospholipid/protein ratio comparable with that of the original membrane.

Recombinations carried out with individual phospholipids from human erythrocyte membranes confirm the notion that opposite charges of lipids and proteins are essential for the initial interaction. Additional confirmation could be derived from recombinations above pH 5.5 with negatively charged proteins and positively charged liposomes containing stearylamine. In addition, it appeared that once the recombination had been formed at pH 4 addition of sodium chloride or change in pH from 4 to 7 was ineffective in splitting the complex. On the other hand no recombination could be obtained in the presence of sodium chloride (0.2M). The extractability patterns of lipids from the