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Short Communications

Some observations on the extra-protein of cross-striated muscle

SZENT-GYÖRGYI *et al.*[1] reported that when glycerated myofibrils are extracted with KCl solutions of high ionic strength with added $MgCl_2$ and ATP or pyrophosphate, myosin passes into solution together with another fraction which is soluble at low ionic strength.

VILLAFRANCA[2] studied the same fraction further, which he called "Extra-Protein", both in glycerated and fresh myofibrils. He suggested that it is another fibrous protein, which in addition to myosin, actin, and tropomyosin, is leached out of myofibrils when they are treated with any medium suitable for the extraction of myosin. He pointed out that its viscosity is reduced by increasing the ionic strength of the solution, but its intrinsic viscosity is lower than that of tropomyosin under the same conditions. Moreover, it is precipitated almost completely at lower $(NH_4)_2SO_4$ concentrations than tropomyosin.

While studying myofibrillar proteins in vitamin E-deficient rabbits, we also observed that a part of the protein material, which is extracted by concentrated KCl, is soluble at low ionic strength, but our results indicate that it is not a pure protein and that some of its properties differ from those reported by VILLAFRANCA.

The purpose of this note is to give a short account of our observations in normal rabbits, to which the observations in rabbits under pathological conditions will be related.

Myofibrils were prepared from fresh muscle of normal rabbits by the method described by PERRY AND GREY[3], slightly modified, and were extracted with high ionic strength buffers (0.5 *M* KCl, 0.04 *M* K phosphate buffer, pH 6.2–8.6; in some cases, 0.01 *M* $Na_2P_2O_7$ and 0.001 *M* $MgCl_2$ were added). The extract was separated by centrifugation at 29000 *g* and dialysed 20–24 hours against 0.0325 *M* KCl containing 0.005 *M* K phosphate buffer pH 7.1; the precipitate was removed by centrifugation, and the supernatant concentrated by suspending it at 0° in a dialysis bag in front of a fan. This protein fraction, which is extracted with myosin and actomyosin and is soluble at low ionic strength, did not exceed 7% of the total myofibrillar protein.

Electrophoresis in the Perkin-Elmer apparatus showed that the extract is a mixture of at least three components (Fig. 1), the relative proportion of which varies according to the conditions of extraction: when the extraction was carried out at lower pH, the fastest component was much increased in relation to the others. This component has the same mobility as tropomyosin; the corresponding peak increases when pure rabbit tropomyosin is added to the solution and no splitting can be detected even after prolonged electrophoresis. The viscosity drop in the mixture, on

addition of KCl, is clearly related to the amount of this component.

A solubility curve in $(NH_4)_2SO_4$ of a sample extracted with KCl-pyrophosphate-$MgCl_2$ extends over a range from 20 % to 60 % saturation and clearly shows a break at about 40 % (Fig. 2); most of the material appears to precipitate between 40 % and 60 % saturation; this is the case with tropomyosin.

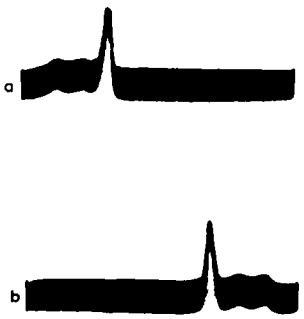

Fig. 1. Electrophoresis of a fraction extracted with 0.5 *M* KCl containing 0.01 *M* Na pyrophosphate and 0.001 *M* $MgCl_2$ and 0.1 *M* K phosphate, pH 6.5. The extract was then treated as described in the text, and finally dialysed against 0.26 *M* KCl containing. 005 *M* K phosphate buffer, pH 6.9. 1.8 mg protein/ml. After 4 h. (a) Ascending boundary; (b) Descending boundary.

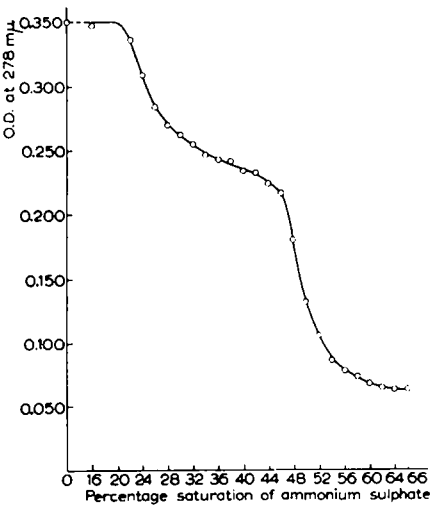

Fig. 2. Solubility curve at pH 7.1 of a fraction extracted with 0.5 *M* KCl containing 0.01 *M* Na pyrophosphate, 0.001 *M* $MgCl_2$ and 0.1 *M* K phosphate, pH 6.5. The extract was then treated as described in the text, and the $(NH_4)_2SO_4$ fractionation carried out at 20° from a solution containing 0.01 *M* KCl and 0.01 *M* K phosphate buffer, pH 7.1. One vol. protein solution (1.8 mg/ml) was mixture with 2 vol. $(NH_4)_2SO_4$ solution of the appropriate concentration.

It appears then that the main component of the protein extracted under the conditions described above is identical with tropomyosin. The other two components have lower mobilities ($3.2 \cdot 10^{-5}$ and $1.6 \cdot 10^{-5}$ $cm^2/V/sec$)* and sometimes appeared to be splitting on prolonged electrophoresis (4–6 h). Their identity is still uncertain. A relation might be suggested with myosin γ (DUBUISSON[5,6]) and protein Y (DUBUISSON[7,8]). The problem is also related to that of adenylic acid deaminase, because the deaminase activity (estimated in 0.225 *M* KCl containing citrate buffer, pH 6.5) of the mixture was found to be quite appreciable, even when tropomyosin happened to be the main component. This is in agreement with the view that deaminase is not identical with myosin (ENGELHARDT[9], NEWTON AND PERRY[10]).

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* These values were estimated in 0.25 *M* KCl containing 0.05 *M* K phosphate buffer, pH 7.4.