A NEW LOW MOLECULAR WEIGHT SUBSTANCE ISOLATED FROM MUSCLE PROTEINS

by

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In connection with analysis of nucleotides and nucleic acids associated with the contractile proteins of muscles a new and hitherto unidentified substance has been isolated. The proteins myosin and actomyosin from skeletal and cardiac muscles have been prepared in a pure state according to earlier descriptions[1,2]. A pure actin is obtained by the same method as described by SZENT-GYÖRGYI[3]. G-actin is prepared according to STRAUB. After addition of salt to the actin solution the polymerised F-actin is spun down in a Spinco centrifuge (4 hours at 40,000 × g). The protein solutions obtained in each of the different cases are then treated several times with chloroform. The protein is denatured and precipitates, and the substances under investigation remain in the water phase. It is possible to precipitate the nucleic acid in acidified 67% alcoholic solution. The low molecular weight substances are then separated on an anion exchanger (Dowex-2) and analysed.

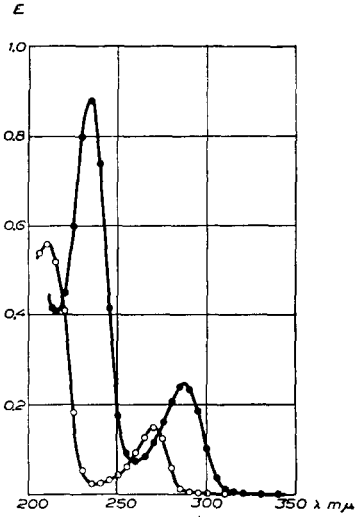

Fig. 1. Ultraviolet absorption curve for the new substance.

Open circles: in 0.01 *M* HCl.
Full circles: in 0.01 *M* KOH.

Among these substances one is of special interest. It is not a nucleotide but rather seems to be an aliphatic compound. It occurs in rather small quantities and has been localized with actin and the actin part of the actomyosin complex. It can be identified in the preparations by its characteristic ultraviolet absorption spectrum, which is shown in Fig. 1. The absorption curve has two maxima; in 0.01 *M* hydrochloric acid at 210 and 270 mμ and in 0.01 *M* potassium hydroxide at 235 and 287 mμ. This spectrum differs distinctly from those of the nucleotides and nucleic acid constituents.

The substance is very difficult to handle because of its lability. It has, therefore, not been possible to prepare it in a pure state and so enable the determination of its specific or molecular extinction coefficient. Nevertheless, it is possible to make a rough estimation of the amount of material in a preparation. Thus polymerised actin (dry weight) seems to contain 1–2% of this new compound.

We do not know the significance of this compound, but it may have something to do with the polymerisation of actin. According to an observation by MOMMAERTS[4], actin can exist in the polymerised form even when all ADP has been dialysed away. Our own experiments have shown that a dialysed, pure F-actin solution gives no ADP when treated with chloroform as mentioned above. Only this new substance is obtained. Since it is rather firmly bound to actin, it may be a prosthetic group of actin.

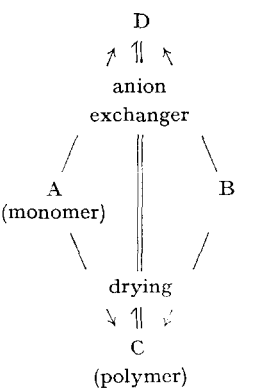

Fig. 2. Different forms of the substance with respect to the absorption of ultraviolet light.

As said before, the substance has a characteristic ultraviolet spectrum. In some cases, however, the substance is altered so that it shows no or at least only a very faint absorption of ultraviolet light. That we are dealing with the same substance is obvious, since it is possible to reverse the alteration in absorption and obtain the original spectrum again. In this way at least three forms, differing with respect to their ultraviolet spectra, are obtained from the muscle proteins (A, B and C in Fig. 2). Some data indicate that the substance has a tendency to polymerise. Therefore the spectrum shown in Fig. 1 is believed to represent the monomer, and the other forms are different degrees of polymerisation. In two of the forms, A and B, the substance reduces Fehling's solution. Besides the

polymerising tendency, the reactivity of the substance is shown by the behaviour on the anion exchanger. Using a fresh column the monomer is eluated in a quite different spectral form than that shown in Fig. 1. This form is denoted as D in Fig. 2. Also the supposed polymeric forms give the new spectrum after elution. It has been shown that some of these reactions depend on a combination of the molecule with metal ions. Thus it seems to be a parallelism between these reactions and the polymerisation of actin, which is dependent upon the presence of bivalent cations.

There are many interesting features about this substance, but it is necessary to know much more about it in order to give a full explanation of all these reactions and to make a study of its role in muscle contraction. This investigation is going on, and a more detailed description together with more results will be published later.

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INTRACELLULAR DISTRIBUTION OF FUMARASE AND ACONITASE IN YEAST

by

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HIRSCH[1] has reported recently that fumarase and aconitase are mainly associated with the non-sedimentable fraction of cell-free yeast extracts. These extracts were prepared from a pure culture of *S. cerevisiae* by mechanical shaking with glass beads at 560 cycles/min. The period of shaking was unspecified, but about 1 hour seems to be the practice in that laboratory[2].

From our studies (to be published shortly) on dehydrogenases in cell-free yeast extracts (prepared by mechanical shaking at 5,600 cycles/min for 10–90 secs), we found that the distribution of certain dehydrogenases varied with the disintegration period: for very short periods, even the "soluble" dehydrogenases (ethanol, malic and lactic) had high activities in the granular portion of extracts. With increasing disintegration periods, these enzymes tended to migrate to the non-sedimentable supernatant.

Similar experiments were done with fumarase and aconitase, because it seemed possible that HIRSCH's failure to observe high activities for these enzymes on the granules was due to excessive disintegration periods. Cell-free extracts prepared by 10, 30 and 90-sec shaking[3] of commercial baker's yeast were fractionated as follows: 30 min centrifugation at 3500 *g* gave "heavy" granules. Recentrifugation of the supernatant for 30 min at 10,000 *g* gave "light" granules. Both sediments were washed once with 25 to 50 vol. 0.9% KCl. Fumarase and aconitase activities were measured spectrophotometrically[4]. Specific activities (increase in optical density at 240 mμ/min/mg dry wt. $\times$ 1000) were measured at room temperature (about 20°) and pH 7.0, with 10^{-2} *M* substrate. Our values multiplied by 10 can be compared approximately with those of HIRSCH.

Both types of granules have high fumarase and aconitase activities when prepared from 10-sec extracts (Table I). 90-sec granules possess almost no measurable aconitase or fumarase activity. Since the acceleration of a mechanical shaker is proportional to the square of the number of cycles/min, our machine is about 100 times more efficient than that used by HIRSCH. Our 90-sec granules are therefore roughly equivalent to 150-min granules prepared on the slower shaker.

Table I also shows that, even when washed with 0.9% KCl, considerable amounts of both enzymes are detached from the granules in the short time required for washing. Therefore, in extracts prepared by long-term shaking, the activities of the resulting granules would be further lowered.

The very high activities of granules obtained after 10-sec disintegrations make it probable that in the intact yeast cell much of the fumarase and aconitase activity is associated with intracellular