ON THE ACTOMYOSIN AS A PROTEIN COMPLEX

by

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The problem of the contractile substance in muscle offers some interesting features as an example of a protein composed of smaller units. SZENT-GYÖRGYI *et al.*[1] could easily split the contractile substance, actomyosin, into two different proteins, crystallized myosin and actin.

It is possible to show that under certain given circumstances the crystallized myosin behaves as a well defined protein. Purified with ammonium sulphate it shows sedimentation and electrophoretic patterns which indicate the presence of one component[2, 3]. Even salting out curves made at 0° C according to DERRIEN[4] indicate that there is one component[5].

The substance is, however, very labile and especially at greater dilution it begins to become polydisperse, so one is obliged to regard myosin as a protein composed of several units. As SZENT-GYÖRGYI *et al.*[6] have shown, it is possible to dissociate several enzymatic functions from myosin by treatment with 0.05 M KCl. Experiments show that only small quantities of material can be washed out. In order to obtain material for physico-chemical studies the solutions must be concentrated to a very large extent[7]. The number of the enzymatic proteins (SZENT-GYÖRGYI's protins) cannot be great.

The substance left behind after washing out the protins was called the skeleton substance by SZENT-GYÖRGYI. It seems that experiments in which a large part of the myosin breaks down in smaller fragments must depend upon a decomposition of the skeleton substance which is the main part of the material in the myosin molecule. Thus experiments with myosin treated with urea for some time give a polydisperse substance, mostly containing material with the sedimentation constant 2–3 in the ultracentrifuge[2]. Experiments with uterus myosin show that the myosin can be broken down completely without any severe treatment, forming polydisperse material with a low sedimentation constant[8]. All these experiments show that a degradation of the skeleton substance has taken place.

Some other experiments indicate that the skeleton substance may be built up of smaller units, probably linked together by hydrogen bonds. BAILEY[9] has found that tropomyosin which can be obtained from muscle, and which is considered by him to be a precursor to myosin, can aggregate from the smaller units of which it consists to form long chains under certain conditions. In the electron microscope crystallized myosin was also always found to polymerize into long chains in not too high concentrations of salt[10]. Pictures of individual molecules have not been obtained, because they seem to be unstable on the object surface. Myosin appears to be a substance with fibre structure as is indicated by the X-ray patterns[11]. The myosin is, however, modified

References p. 58.

through the attached proteins and thus in certain cases it can behave as a globular protein. X-ray investigations have hitherto failed to give any information concerning the nature of the contraction[12]. Perhaps this results from the fact that the crystalline part of the fibre structure is unaltered. The small members of protins play a dominant rôle in the process of folding the chain and where they are attached the fibre is less well organized from X-ray point of view.

Actin in polymerized state is also a kind of fibre protein, but not the same kind as myosin. In this case the units, G-actin, are probably bound together by prostethic groups[13]. It has also been impossible to obtain G-actin in a monodisperse state. It may be either that the G-actin is damaged a little by the method of preparation or, as the electron pictures indicate, that the actin molecules in the chain differ a little in shape. Each unit has an average molecular weight of 150000 as judged by ultracentrifuge determinations[14]. Actin polymerized at p_H 7 consists of at least 1000 such units, so the molecular weight of this F-actin must be of the order 10^9, while still being soluble. So G-actin is a molecule from which higher units can be formed.

Another case in which the protein molecules can react and build up higher units is the reaction between myosin and actin to form actomyosin. Myosin and actin react here in a stoichiometrical way[15], and the new molecule has a number of new properties. The link between myosin and actin does not seem to be of acid-base character and in this bondage SH-groups play a part[16]. Protins of different kinds in the myosin seem to be the agents connecting myosin and actin. If this is the case a definite number of them is attached to every myosin molecule as is shown by the stoichiometrical relationship.

Another question which has been much discussed in connection with the actomyosin problem is how ATP can occur in muscle, as it has been found, without reacting with actomyosin. The reaction between these compounds only takes place when a certain impulse for the reaction is given. SZENT-GYÖRGYI has shown the importance of the potassium ion concentration for the reaction. HERMANN[17] has shown that myosin in $CaCl_2$ (or $MgCl_2$) solutions does not adsorb any ATP while myosin in solutions of KCl with a smaller amount of $CaCl_2$ does adsorb ATP. In this case the adsorption curve has a sharp maximum at about 0.1 M KCl, when the amount of $CaCl_2$ in the solution is 0.0005 M.

An electrophoretic investigation has shown that the Ca (or Mg) ions are firmly connected to the myosin in solutions of $CaCl_2$ (or $MgCl_2$)[3]. The myosin had completely lost its acid character. It is possible to show that the Ca (or Mg) ions bound to myosin are liberated again when KCl is added. The link between Ca (or Mg) ions and myosin seems to be of permutite-like nature.

BUCHTAL has shown that the ultraviolet spectra of the purine group are altered when ATP is adsorbed on the myosin. There must certainly be some kind of dipole-dipole or dipole-ion bond between the purine end of the ATP molecules and the groups which can even bind Ca-ions. The Ca-ions block these groups, preventing a linkage with ATP and the groups bind Ca-ions more strongly than ATP. The ratio K-ions to Ca-ions regulates by means of the permutite equilibrium the number of groups free to bind ATP.

Finally we wish to remark that myosin, F-actin and actomyosin belong to a part of protein chemistry that has not yet been systematically classified, a part which deals with the definite compounds existing between protein molecules. These compounds can have quite new properties and certainly constitute the structure elements in many tissues.

References p. 58.

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SUMMARY

Crystallized myosin is a well defined product. SZENT-GYÖRGYI has shown that it is composed of several proteins (protins and skeleton substance). The number of protins in every molecule cannot be large and as myosin is a substance of definite molecular size their number also ought to be definite. The skeleton substance (the fibre) which is the main part of the molecule, is built up of smaller units.

The ATP molecules are bound to the molecule at certain points which can be blocked by Ca (or Mg) ions. The ratio Ca/K regulates the amount of ATP molecules that can be bound.

Myosin, F-actin and actomyosin are composed of protein molecules built up in a definite manner as other common compounds are built up of atoms.

RÉSUMÉ

La myosine cristallée est un produit bien défini. SZENT-GYÖRGYI a montré qu'elle est composée de plusieurs protéines (protines et substance de squelette). Le nombre de protines dans chaque molécule ne peut pas être grand et vu que la myosine est une substance de dimensions moléculaires definies, ce nombre doit pouvoir être défini lui aussi. La substance de squelette (la fibre), qui constitue la plus grande partie de la molécule, est formée par des unités plus petites.

Les molécules d'ATP sont liées à la molécule de myosine en certains points, qui peuvent être bloqués par les ions Ca^{++} (ou Mg^{++}). La relation Ca/K régit le nombre de molécules d'ATP qui peuvent être liées.

La myosine, la F-actine et l'actomyosine sont édifiées par des molécules de protéines, liées ensemble d'une façon définie, tout comme les composés ordinaires sont constitués par des atomes.

ZUSAMMENFASSUNG

Kristallisiertes Myosin ist eine wohl definierte Substanz. SZENT-GYÖRGYI hat gezeigt, dass es aus verschiedenen Proteinen (Protinen und Gerüstsubstanz) zusammengesetzt ist. Die Anzahl der Protine in jeder Molekel kann nicht gross sein, und da Myosin eine Substanz von bestimmter Molekelgrösse ist, so sollte diese Anzahl auch bestimmt werden können. Die Gerüstsubstanz (Fiber), die den Hauptteil der Molekel ausmacht, ist aus kleineren Einheiten zusammengesetzt.

Die ATP-Molekeln sind mit der Myosinmolekel an gewissen Stellen verknüpft, die durch Ca^{++} (oder Mg^{++})-Ionen blockiert werden können. Das Verhältnis Ca/K bestimmt die Menge der ATP-Molekeln die gebunden werden können.

Myosin, F-Aktin und Aktomyosin bestehen aus Proteinmolekeln die in bestimmter Art aufgebaut sind, gerade wie gewöhnliche Verbindungen aus Atomen aufgebaut sind.

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