ENZYMOLOGY AND MECHANOCHEMISTRY OF TISSUES AND CELLS

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The subject which it is intended to discuss here may be regarded as a particular case of the biological problem of much wider scope, namely that of the role of enzymes as integral components of elementary physiological mechanisms. Only one aspect of this problem will be considered here, that of the enzymatic factors of the function of biological motility. At first applied to muscle, the same approach could be extended subsequently to almost all known forms of biological movement.

For obvious reasons it is with muscle that we begin. Adenosinetriphosphate (ATP) has long ago been recognized as the immediate source of energy for the performance of the work of muscle. Retrospectively it appears puzzling that for a considerable length of time no attempt has been made to investigate the nature of the interaction of ATP, as bearer of potential chemical energy, with the contractile substance of muscle, its structural proteins. The study of muscle activity proceeded along two independent, completely separate lines. One of these lines was the study of the metabolic processes, wich finally provide the energy for muscular contraction. The brilliant work of Meyerhof and Parnas, Joseph and Doroty Needham, Carl and Gerty Cori, their teams, and numerous other investigators had led to a detailed knowledge of the metabolic processes in muscle. This knowledge appeared almost exhaustive, and its main result was the above-mentioned recognition of the role of ATP in the energetics of muscle.

The other line of research was the study of muscle proteins. Danilewsky in Russia, Kühne in Germany were among the first to attack this field. Of fundamental importance were the investigations of Edsall and Muralt, and of Weber about the properties of myosin, unanimously regarded as the predominant component of the contractile mechanism of the muscle fiber. Later the discovery of actin by Straub was another significant step in this field.

To everyone engaged in the study of muscle it has always been evident that the work of muscle is the result of interaction between low-molecular, crystalloid products of the metabolic changes going on in muscle, with the macromolecular protein substances which constitute the mechanical framework of the muscle fibrill, its contractile mechanism. This statement is almost a truism. Nevertheless, as already mentioned, the two lines of study of muscular activity developed independently, as if separated by a deep gap. The possibility of overbridging this gap, of bringing the two lines of approach to a close contact, arose from studies of enzymological character. The enzyme, adenosinetriphosphatase (the conventional name, ATPase will be used) was found to represent the link which connects the biochemical and the mechanical events, responsible for muscular contraction.

Once this connecting link was revealed, it became possible to speak of "m e c h a n o c h e m i s t r y" as a new sphere of research, where mechanical and chemical phenomena are studied in their mutual interrelations, under the different aspects, associated with the performance of mechanical work, as during muscular contraction, or with the various other forms of movement in biological objects.

ATPase is the enzyme which splits adenosinetriphosphate and liberates the chemical energy, accumulated in its high-energy (macroergic, as we call them) phosphate bonds. Thus the motive force is furnished for the performance of mechanical work. The study of this enzyme, which we undertook, in collaboration with Dr Liubimova, led to the unexpected discovery that the enzymatic activity belonged to myosin, the protein which constitutes the basis of the contractile substance of muscle. On other words, the contractile substances catalises itself the reaction which yields the enrgy for the contraction.

The logical development of these studies was to investigate whether ATP, the substrate of the enzymatic activity of myosin, might not produce some changes of the physical properties of myosin itself, and thus lead to the mechanical effects, associated with the processes of contraction or relaxation.

Experiments of this kind were made almost simultaneously in two different geographical points - in Moscow and in Cambridge, and the problem was attacked on different levels. Our group in Moscow (Engelhardt, Liubimova and Meitina) used macro-systems - myosin threads as models of muscle fibers. The experiments of the Cambridge group (Joseph and Doroty Neddham,Dainty, Kleinzeller, Shi Chang-Shen and Lawrence) were on the molecular level - with myosin solutions. It is on these two levels that the whole further research on the biochemistry of muscular contraction, - or, as we may call it, on the mechanochemistry of muscle and other objects, -proceeded and is still going on.

The results of the very first experiments were clear-cut and unambiguous. In both cases, in completely different manners, profound changes of the physical properties of myosin were observed under the influence of ATP.

In the experiments of the Cambridge group the effect consisted in an instanteneous drop of viscosity of the protein solution, indicating profound changes of molecular shape of the protein particles. This effect deserves to be designated in the beiochemical literature as the "Needham effect", because it is one of the most conspicuous effects in the field of mechanochemistry, and has played a decisive role in the study of muscle proteins, for the discovery of actin in particular. On the other hand the experiments with myosin threads initiated a series of.numerous investigations in which different kinds of "muscle models" were used, of increasing perfection and complexity: oriented or dehydrated (glycerinated) myosin threads, glycerinated muscle fibrills or strips, whole muscle preparations.

Two features of the mechanochemical machinery, as it appears from the above-mentioned results, deserve to be specially pointed out. First, the enzymatic nature of the contractile substance, and second - the reciprocal character of the interaction of enzyme and substrate in this case. The fact that myosin, when reacting with ATP as its substrate not only produces a change of the substrate (as all enzymes do), but at the same time itself undergoes characteristic changes of its properties, promted Needham to propose the admirable designation of myosin as a "contractile enzyme". And consequently it became possible to regard muscular contraction as being "essentially an enzyme - substrate combination". We now may say that these results were due to lucky chance, namely, that at that time the methods of fractionation and purification of muscle proteins were not as elaborated as they are now. In fact, with highly purified myosin the experiments would have been negative, for it is only when myosin is combined with another substance (in muscle - with the other protein, actin, in the form of actomyosin) that directly observable changes of physical properties are produced by ATP.

When we "purify" myosin, separate it from actin, its properties are changed: the catalytic activity remains unaffected, myosin is still an enzyme, but the contractility is lost, myosin is no more a "contractile enzyme". This statement should not be taken too rigorously. Some of our own observations, with Dr.Kafiani, when threads of compressed surface-spread myosin monolayers, according to Hayashi, were used, as well as unpublished results of Dr.Cheeseman, in London, who studied area /pressure diagrams of surface spread myosin, both point to the possibility that even highly purified myosin, free of actin, still posseses a certain degree of physical susceptibility towards ATP.But this question is still far from being settled/ and as it is of minor importance for the present discussion, it can be left without further consideration, and the terms "myosin" and "actomyosin" will be used here in most cases indiscriminately.

Evidently other factors, as for instance ionic effects, also take part in the complex phenomena of muscular activity or biological motility in general. But these are beyond the scope of the present discussion and their nature is far from being sufficiently understood. But apart from the enzymo-substrate type of reactions there is another important kind which must be mentioned here. Whereas the enzyme-substrate reactions represent interactions of low-molecular substances with macromolecular partners, the other type of reactions, which seem to play an important role in mechanochemical phenomena, are those where macromolecular compounds interact with one another.The prototype of such reactions has already been mentioned - it is the reaction between myosin and actin, the formation and dissociation of actomyosin. As will be shown later, there is already some evidence,that other substances as well may form complexes with myosin, influencing its properties in a manner which closely resembles the effect observed when myosin reacts with actin.

Returning to the purely enzymological side of the problem, it must be stated, that the question whether myosin itself is the ATPase, or the enzyme is a separate entity, only bound to the contractile protein has been discussed lively and during a considerable lenght of time. The main objection seemed to be based not on convinving experimental evidence but rather on a precenceived opinion which could be shematically formulated thus: "There is so much myosin in muscle that it cannot be an enzyme". The logic of the argument is of the same kind as if one would insist that haemoglobin cannot be a catalytically active protein because there is too much haemoglobin in a blood cell. The verdict of time,that most exacting judge in matters of science, has been decidedly in favout of the identity of myosin and ATPase. The evidence has been of two kinds, preparative and functional.

All attempts to obtain preparatively from myosin some fraction where the enzymatic activity would be concentrated and the other characteristic properties of myosin (for example its actinecombining property) would be absent, have failed. Taking into account the difficulties and limitations of the yet available methods of fractionation and separation of proteins, only positive results are decisive. The value of negative results, that is of the failure to separate a presumed mixture into its components are of very limited value. Therefore much more weight has to be attributed to less direct, but in this case more reliable, functional evidence, based on the parallelism, or, on the contrary, independence of the changes of the properties of the presumed separate components of a complex under as different conditions as possible. Evidence of this kind is available, from different laboratories.

Bailey and Perry were the first to show an almost exact parallelism of the changes of ATPase activity and actin-binding property of myosin preparations treated with the thiol poison, iodosobenzoate.

My collaborator, Dr Iarovaia, in the Moscow University, carried out similar experiments, using a wider range of factors, affecting the enzymatic activity of myosin - heat denaturation, pretreatement at different pH, action of cadmium and silver ions. An almost exactly identical behaviour of enzymatic activity and of actin-binding property was found. In the work of Mrs Venkstern in our laboratory photochemical changes of myosin have been studied, and here again a parallelism between loss of enzymatic activity and decrease of the actic-binding capacity was observed.

It would be a very strained interpretation indeed to regard this as a mere coincidence, and to ascribe to two separate proteins such a completely identical behaviour toward so widely differing factors as temperature,pH, thiol poisons, photochemical action. The conclusion can only be drawn that both properties belong to one and the same protein. It is for this protein, possessing the ATPase activity and the property to combine with actin that the name of m y - o s i n in the strict sense of the word should be reserved.

Not only in the intact molecule of myosin, but even in the primary products of its breakdown by a mild action of proteolytic enzymes, in the "meromyosins" of Andrew Szent-Györgyi, both abovementioned properties continnue to be associated with one and the same fraction.

The mechanochemical effect- changes of physical properties under the influence of ATP, - depends strictly on two conditions: the enzymatic activity must be maintained, and myosin must be combined with a partner which imparts the necessary physical reactivity. In muscle the role of this partner belongs to actin. But under experimental conditions actin appears not to be the only substance which can produce the said effect.

In suspensions of actomyosin gel or of finely dispersed muscle fibrils ATP produces a marked effect of syneresis: the particles shrink and when they are centrifuged the volume of the sediment is considerably reduced; actin-free myosin gel suspension does not show this effect. But it has been shown by Ashmarin in Leningrad, that if mayosin is treated with certain dyes, for instance congo-red, the compound myosin-dye behaves exactly in the same manner as does the complex myosin+ actin.

Synaeresis is not a phenomenon to which we could attribute any significant role in the processes of muscular activity; and dyes are evidently far from being agents of biological nature. Much more importance has therefore to be attributed to recent experiments of Dr.Vorobjev, one of my former pupils, in Leningrad. By adding native, non-depolimerized nucleic acid to a myosin solution, Vorobjev obtained from such a mixture threads (we may call them "nucleo-myosin threads") of considerable strength, which when extended by a load would contract anisodimentionally under the action of added ATP, lifting the load and thus performing mechanical work. This behaviour of the nucleo-myosin thread resembled in all details that of glycerinated actomyos in threads extensively studied by Weber and his associates. The improtant point is that the characteristic effect is observed only as long as the enzymatic, ATPase activity of myosin remains unimpaired. Myosin soluticns which have lost their ATPase activity will not yield mechanochemically active threads. Presence of calcium ions, which are known to be indispensible activators of ATPase is also necessary for the mechanochemical effect; in presence of the chelating agent, EDTA (ethylene-diaminotetraacetate) which removes Ca ions, the threads do no more contract cn additicn of ATP; on the contrary, they begin to extend under the load, because now the plastifying effect cf ATP, found by Weber, becomes apparent. On addition of an excess of calcium, surpassing the molar concentration of EDTA, the ATPase activity is restored, and the thread begins again to contract on addition of ATP. This demostrates unambiguously the fundamental role of the enzymatic property for the appearance of mechanochemical reactivity. Certain facts, which will be mentioned a little later, seem to indicate that the formation of nucleomyosin is perhaps not merely an experimental trick, but may actually take the place of actomyosin formation in cases where actin is absent in a certain kind of cells.

The nature of muscular activity is a vast and extremely multiform problem, where morphology and biochemistry, thermodynamics and enzymology, energetics and colloid chemistry are closely interconnected. I have - admittedly arbitrarily,- selected a few aspects among this multitude, and outlined them in a most schematic way. Muscle is the object, in which living Nature has reached the utmost perfection in the transformation of chemical energy into mechanical work and movement. But motility is one of the fundamental, most universal manifestations of life. Its forms are very diversified: purely protoplasmic flow, as observed in myxomycetes; ameboid movement; intracellular movement during mitosis; movement of spirillae, flagellate bacteria, protozoa, animal and plant sperm cells; eventually rapid movement is observed in higher plants, for example in insectivorous species of Mimosa pudica; and finally in the animal kingdom all the diversity of muscular movement, from the very slow motion of plain muscles to the enormous velocity in an insect wing.

The question can be raised: Nature elaborated completely different fundamental mechanisms to produce each of these very different forms of motion? Or are all these forms of movement based on some common, elementary principles? At first sight it may appear hardly probable that such profoundly different types of motion, as for example flaggelar and muscular, have anything in common from the biochemical point of view. But it is exactly in the field of biochemistry that the idea becomes more and more evident that elements of far-reaching unity are encountered along with the almost boundless diversity of living forms. Throughout the animate kingdom, to its very extremes, from a bacterial cell to the brain tissue of man, we find operating fundamentally identical metabolic mechanisms, such for example as that of cellular respiration, with all its complicated system of enzymes and coenzymes, intricate cyclic reaction sequences, extremely refined accompanying phenomena responsible for the accumulation of energy of oxydative processes in the form of high energy phosphate compounds, and so on.

If this "unity among diversity" is firmly established in the field of biochemical dynamics, it is not unreasonable to expect that the same principle can be encountered in the field of biological kinematics, in the sense that all the diverse forms of biological motility have similar, common to all of them, fundamental biochemical mechanisms. During the last decade considerable experimental evidence in favour of this assumption has accumulated, and it is intended to give here a brief review of it.

But first it will be necessary to summarize the criteria which can help us to decide whether similar mechanisms to the one of muscular contraction are involved in the other forms of motility.

Schematically these criteria are the following:

1) Analytical demonstration of ATP in the motile objects, and cessation of movement as soon as the store of ATP is exhausted.

2) Demonstration of the presence of proteins, with properties resembling those of the "classical" contractile substance, namely myosin or actomyosin. The properties to be looked for would be: a)susceptibility to physical changes (viscosity,birefringence) under the influence of ATP;

b) contraction effects produced by ATP

c) ATPase properties of the protein.

3) Demonstration of kinematic effects, produced by the application of ATP from the outside to the objects in their native state or after mild treatment (glycerination)

4) Demonstration of the presence of a sufficiently high ATPase activity, and correspondence of the level of activity with the motile efficiency.

Obviously, these different criteria are not equally reliable, especially when taken separately. The conclusions become much more definite, if several independent criteria can be satisfied.

With these considerations in mind, the question can be discussed now, what experimental evidence is available, concerning the basic principles which govern the function of motility in the different biological objects.

Sperm cells

Spermatozoa have been the object of our studies, carried out in collaboration with Dr. Burnasheva (some of the earlier result have been brierly mentioned in a review article in the "Advances in enzymology). The sperm cells contain appreciable amounts of ATP. Under favourable conditions (aerobiosis, presence of glucose) the ATP level remains constant over long periods. If the cells are deprived or glucose and their respiration is stopped by removal of oxygen or by cyanide poisoning, a steady decrease of the ATP content is observed, and at the same time the motility falls. As soon as the ATP store is exhausted the cells become motionless. When normal conditions are restored, ATP is synthetized in the cells, and at the same time motility reappears.

By treating sperm cells with solvents, used for the extraction of myosin from muscles, a protein preparation has been obtained, which resembled in several respects myosin. The protein, for which the name "spermosin" was proposed, has similar with myosin solubility properties, and possesses an appreciable ATPase activity.

Burnasheva succeded in an elegant way to demonstrate that both spermosine and the ATPase activity are localized in the motile part of the sperm cell, in its tail. By exposing spermatozoa to carefully controlled mechanical treatment in a blendor it has been possible to dissect the cells, break off the tails from the nucleus-carrying head of the sperm. The two fractions can readily be separated by differential centrifugation. It was the motile part of the sperm cell, the tail, which contained practically the whole ATPase activity found in the intact cells, and spermosine could be obtained from this same fraction.

Attempts to obtain some kind of mechanochemical effects with spermosin, as they are shown by actomyosin, were at first unsuccessful. But the following experiment permitted to obtain a positive result in this respect also. As already mentioned, myosin, as such, when free of actin, does not show the properties which we designated as mechanochemical reactivity: changes of physical properties under the influence of the energy-bearing metabolic product, ATP. This reactivity appears only when myosin is combined with a certain partner - actin in muscle, a dye in Ashmarin's experiments, nucleic acid in those of Vorobjev. From sperm cells no protein with the properties of actin could be isolated, and the negative results mentioned might have been due to the absence of such an necessary partner for the spermosine isolated from the cells. Now Ivanov in Moscow has shown very clearly that the reaction between myosin and actin is completely devoid of species specificity: myosin from one source will react in exactly normal manner with actins prepared from different animal species. Taking in consideration this lack of specificity of the contractile proteins, Burnasheva examined the possibility of combining spermosin with actin prepared from muscle.

The results almost exceeded our expectations. Addition of actin to the spermosin solution resulted in a considerable increase of viscosity, of the same order as observed in an experiment with myosin. When ATP is added to this mixture, there is an abrupt drop of viscosity, again exactly in the same manner, as observed in experiments with myosin. It is evident that spermosin forms a complex with actin, just as actomyosin is formed from myosin and actin. We may call this complex "acto-spermosin", in analogy with acto-myosin.

No answer is so far available to the question- what substance takes the place of actin in the sperm cell to form the mechanochemically reactive complex with spermosin. The already mentioned experiments of Vorobjev with nucleic acid suggest one possible answer to this question. The sperm cell is extremely rich in nucleic acid. Would not a part of it be used in the cell not as carrier of hereditary information, but as a component of the chemical machinery of the motor apparatus of the cell? But here we have to wait for furhter experimental results.

Attempts to restore the motility of spermatozoa which had become motionless because of the exhaustion of the ATP reserves of the cell by supplying ATP from the outside, in the surrounding medium, remained unsuccessful. In experiments of this kind only positive results are of value, as negative ones may be due to the impermeability of the cell to the energy-beering nucleotide.

This explanation of the negative results of our experiments is supported by observations on other types of living objects, possessing flagellar motion, as carried out by Weber and his associates. They used flagellated protozoa, trypanozomes, and giant sperm cells of the grashopper. The material was treated with carefully selected concentrations of glycerol. This treatement removed low-molecular substances and water-soluble proteins, whereas the structural proteins, responsible for the movement of the flagella, being in the gel state, remained undissolved. At the same time the permeability of the surface layers is profoundly changed, so that the contractile mechanism becomes easily accessible to ATP, applied from outside. Sich preparations, designated as "flagellar models", perform regular movements when ATP is applied, even in the case when the flagella are broken off from the body of the cell. As a pretreatement with glycerine was neccesary to obtain this effect, it may be concluded that the negative result in our experiments was actually due to the presence of a permeability barrier, which prevented the access of ATP to the essential parts of the contractile apparatus of the flagellae.

Summarizing the results obtained in the study of flaggelar movement, it can be stated that in this case almost all of the criteria enumerated above are satisfactorily met. A far-going similarity of the basic enzymatic and mechanochemical factors of this type of motility and that of muscular contraction may be regarded as firmly established.

Even the most primitive form of biological movement, namely the flow of protoplasm in myxomycetes displays certain features in common with muscular motion. Fibrillar protein, resembling in properties myosin, has been described in these organisms, and ATP is involved in the processes of protoplasmic flow. Of great interest are the contribution of Weber's group, in which different forms of cellular movement have been extensively studied. Ameboid movement, contraction of fibroblasts, and even the most refined processes of displacements of the chromosomal apparatus during the mitotic cycle have been shown in these investigations to be in many respects closely similar to muscular contraction, although the velocity differs by about three orders. Preparations of contractile proteins have been obtained from motile cells, by methods similar to those used for the isolation of myosin from muscle. These proteins resembled actomyosin, as their viscosity decreased after addition of ATP, and they possessed ATPase activity; in gel form the proteins contracted when treated with ATP. Moreover, glycerinated "cellular models" have been prepared, which reacted by specific transformations on addition of ATP The authors advanced the view, that from these primitive froms of biological motion the highly specialized muscular mechanism has been developed in the course of evolution.

Finally I will venture to make a step still further, and leaving objects of animal origin, mention the observations of my collaborator, Poglasov, who experimented with higher plants. Here only one of the criteria which I mentioned has been so far followed up, namely the distribution of ATPase activity [illegible] -

examined in a variety of plants, possesing motility and devoid of this property.

Mimosa It was found that the leaves of Mimosa pudica posses a high ATPase activity.Towards autumn or when kept in unfavourable conditions in the laboratory the plants gradually loose their motor reaction - the rapid folding of leaves when touched, and this was accompanied by a marked decrease, sometimes almost complete disappearance, of the enzymatic activity. Numerous other plants that have been investigated, which do not possess the motile function,exhibited very low, often scarcely detectable ATPase activity of the leaves. Even closely related species, such as Acacia,belonging to the same family, had hardly perceptible activity. Only in one variety, which did not display mobility, an appreciable ATPase activity of the leaves was found, but still consirably less than that observed in Mimosa pudica. Perhaps we have to deal here with a kind of rudiment, in the sense that the enzymatic activity has remained while some other link of the motility mechanism was already lost.

Attempts to isolate some specific protein from mimosa leaves, which could be regarded as responsible for the motile function, - some hypothetical "mimosin", - have so far failed. This is not astonishing, as the extraction of proteins in their native state from leaf material is known to be a difficult task, often hardly possible at all and usually involving rather drastic treatement; and one of the most characteristic properties of contractile proteins, at least those of animal origin, is their extreme lability.

We clearly realize that the information obtained on plant material is as yet very poor. Perhaps I may close my lecture with an invitation to our colleagues living in warmer countries, and having at their disposal a much more numerous and more readily accessible choice of ~~vegetable~~ Botanical objects, to undertake corresponding experiments. Perhaps the idea of "unity among diversity" could thus obtain further support.

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