Short Communications and Preliminary Notes

STOICHIOMETRIC AND DYNAMIC IMPLICATIONS OF THE PARTICIPATION OF ACTIN AND ATP IN THE CONTRACTION PROCESS*

by

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The present communication summarizes some physiological consequences of recent studies concerning the role of ATP in the molecular transformations of actin. It is assumed that the polymerization and depolymerization of this protein take place in the contractile cycle (SZENT-GYÖRGYI[1]; PARRISH AND MOMMAERTS[2]).

As has been shown in this laboratory by the study of pure actin preparations[3,4], the polymerization of actin is associated with a stoichiometric binding and dephosphorylation of ATP to ADP, in such manner that 1 mole of the nucleotide is decomposed by the reaction with about 60,000 grams protein***. If muscle contains 25 to 30 mg of actin per gram[5], the reaction *in vivo* would imply the dephosphorylation of 4 to $5 \cdot 10^{-7}$ mole ATP per gram muscle. This is the same amount of nucleotide as has been found to participate in one twitch of this amount of muscle[6,7].

While for the reversible depolymerization of actin *in vitro* ATP has to be provided by the experimenter, it is assumed that *in vivo* the bound ADP is rephosphorylated by a phosphate donor system. Repeated polymerization and depolymerization in tetanic activity will then lead to a steady state of breakdown of energy-rich phosphate.

The physiological rate of dephosphorylation has been estimated for a few cases[7]; for frog *sartorius* at 20°, it amounts to a conversion of ATP to ADP at a velocity of $3 \cdot 10^{-4}$ mole per gram muscle per minute, or of $2 \cdot 10^{-4}$ for the *gastrocnemius*, whereas a rate of 10^{-3} mole per gram per minute appears to be a representative average for mammalian muscle, active *in vivo*.

Such tetanic activity does not consist of a repetition of full twitches, but is a sequence of incomplete, and incompletely reversed, primary processes[8]. The "degree of completeness" has been defined and estimated by the author elsewhere[8].

It is found that for a frog *sartorius* at 20°, stimulated at a rate of 180 cycles per second in the experiments of HARTREE AND HILL[9], the completeness of each event is 11%, whereas in the work of LUNDSGAARD[10] on the *gastrocnemius* stimulated 50 times(?) per second the completeness is about 14%.

If the steady breakdown of ATP is due to a repeated polymerization of actin and its reversal, at a frequency equal to that of stimulation, and with a completeness as indicated above, the rate of dephosphorylation may be calculated.

For the frog *gastrocnemius*, 50 events per second with a completeness of 14% of the maximal value of $5 \cdot 10^{-7}$ mole per gram would account for a rate of dephosphorylation of $2 \cdot 10^{-4}$ mole per gram per minute. For the *sartorius* under the given circumstances, a value of $6 \cdot 10^{-4}$ mole per gram per minute results. These values agree closely with the actually observed rates of breakdown of $2 \cdot 10^{-4}$ resp. $3 \cdot 10^{-4}$ mole per gram per minute.

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*** The disappearance of ATP and the formation of ADP during the polymerization of crude actinpreparations has been detected by STRAUB[14] resp. LAKI[15].

It must be emphasized that these results are *not* based upon any kinetic aspects of the actin transformations. The rates of polymerization and depolymerization *in vivo* are not known (compare PARRISH AND MOMMAERTS[2]) and would in any case not be a suitable base for calculation due to the discontinuous nature of the events.

As has been pointed out repeatedly by the author (*e.g.*, references[7,11]; compare also BRAVERMAN AND MORGULIS[12]; PERRY[13]), the enzymic activity of myosin-ATPase cannot account for the rate of breakdown of ATP in physiological activity. The present considerations suggest that physiological dephosphorylation is not due to straight enzymic hydrolysis, but is linked with the repeated molecular transformations of actin.

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A STUDY OF AMINO ACID INTERRELATIONSHIP USING SIMULTANEOUS ADAPTATION

by

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The adaptation of a micro-organism for the utilisation of a given substrate involves the simultaneous adaptation for the intermediate metabolic substances derived from this substrate. This logical implication has been used by STANIER[1], KARLSON AND BARKER[2], AJL[3] in the study of oxidation by bacteria.

The same method can be usefully employed in the investigation of amino acid metabolism. In this case one can conveniently measure the growth of the bacteria as the response to the utilisation of a given amino acid. The lag phase can be interpreted, at least partially, as the time necessary for the synthesis of the enzyme involved in the utilisation of a given substrate[4]. In the course of a study on the metabolism of glutamic acid in *B. subtilis* (strain M_1), it has been observed that this bacterium requires one of the following amino acids: glutamic acid, aspartic acid, arginine, ornithine or proline. The relationship between these amino acids is well known[5,6,7,8,9]. One may ask, whether the bacteria use the glutamic acid in replacement of arginine or proline or if these two amino acids are converted into glutamic acid, which is secondarily used as the normal source of nitrogen.

In the experiments reported here, the bacteria are grown in aerated media containing the ordinary mineral salts, glycerol *M*/20, and one of the above cited amino acids at *M*/200. When bacteria, which have been grown on glutamic acid are washed and used as large inoculum in culture