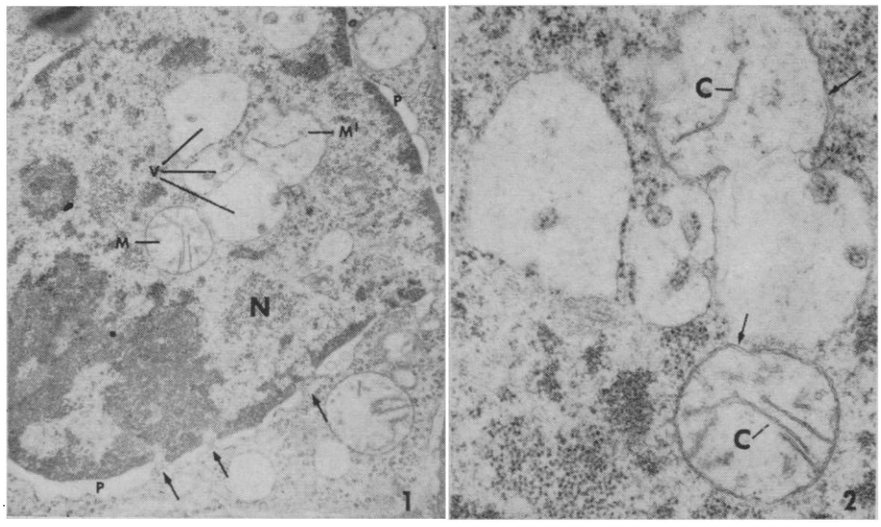


Fig. 1 (left). Portion of L 1210 leukemia cell. The nucleus (*N*) contains a recognizable mitochondrion (*M*), an apparently altered mitochondrion (*M'*), and various undefined vacuolar structures (*V*). The perinuclear cisterna (*P*) shows marked dilations. Prominent nuclear pores are visible (arrows). Glutaraldehyde-osmic acid fixation, Epon embedding, and uranyl acetate counterstaining (× approximately 13,000). Fig. 2 (right). Higher magnification of portion of the nucleus of Fig. 1. Characteristic double membrane (arrows) and cristae (*C*) of the intranuclear mitochondria (*M* and *M'*). Note the absence of nuclear envelope around these structures (× approximately 29,000).

double nuclear membrane. The possibility may then be considered that the "nuclear" mitochondria are derived from such an invagination, and that subsequently the communication between the invaginated area and the general cytoplasm became obliterated and that the portions of nuclear membrane surrounding the trapped mitochondria disappeared. The most likely possibility, however, is to assume that at telophase the mitochondria became trapped within the nuclear envelope as it formed, and this could be considered as an aberration of cell division which may be expected in malignant cells.

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## Mercurial-Induced Transformation of Myosin Prevented by Adenosine Triphosphate and Pyrophosphate

Abstract. *Adenosine triphosphate and pyrophosphate prevent the loss of Ca++-activated adenosine triphosphatase activity caused by high concentrations of mercurial sulfhydryl reagent. They concomitantly prevent the transformation of myosin into faster-sedimenting products. This is adduced as support for the hypothesis that the strategic sulfhydryl group is not binding adenosine triphosphate at the active site, but is initiating a conformational change upon its reaction with the mercurial reagent.*

When small amounts of *p*-chloromercuribenzoate are added to myosin, an activation of the Ca++-activated adenosine triphosphatase occurs; when large amounts of *p*-chloromercuribenzoate are added, an inhibition occurs (*1*). However if pyrophosphate or adenosine triphosphate (ATP) are present before the addition of the mercurial sulfhydryl reagent, the inhibition can be reduced or abolished. Morales and Hotta, using pyrophosphate, found that *p*-chloromercuribenzoate in slight excess of the sulfhydryl content of myosin caused no loss of adenosine triphosphatase activity (*2*). Gilmour and Gellert, using ATP, found a low steady rate of adenosine triphosphatase activity even with such a large excess of *p*-chloromercuribenzoate as 122 moles per $10^5$ grams of protein (*3*). In a manner similar to its protective action against *p*-chloromercuribenzoate, ATP blocks the reaction of iodoacetamide with the sulfhydryl group whose loss destroys the Ca++-activated adenosine triphosphatase activity (*4*).

Gilmour and Gellert suggest that the sulfhydryl group, whose titration causes inactivation, forms part of the ATP-binding active site (*3*). Stracher also believes that the sulfhydryl is at the active site where it is protected from inactivation by the presence of the substrate (*4*). However Morales and Hotta question how the tremendous affinity of mercury for sulfide could be thwarted by pyrophosphate or ATP actually lodged at the sulfhydryl site (*2*).

The alternative hypothesis to this sulfhydryl group being at the active site is that reaction of this sulfhydryl group in a relatively remote location initiates a conformational change which alters the active site. In the range of mercurial titration where loss of

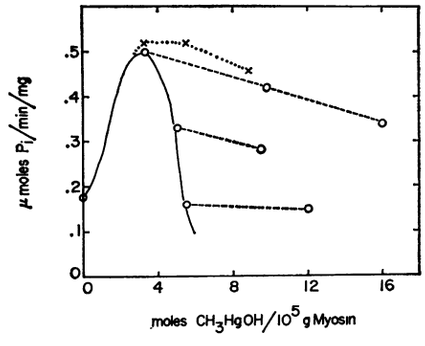


Fig. 1. Effect of methylmercuric hydroxide on Ca++-activated adenosine triphosphatase activity in the presence or absence of ATP or pyrophosphate. Adenosine triphosphatase determinations were performed by *p*H-stat at *p*H 7.0 and 20°C; the zero-order kinetics measured the number of micromoles of base uptake per minute per milligram of protein; colorimetric standardization allowed the data to be expressed as μmoles of inorganic phosphate ($P_i$). The assay system contained 0.25*M* KCl, 5 m*M* Ca++, and 2 m*M* ATP. Addition of pyrophosphate and methymercuric hydroxide to a sample of myosin was made before addition of 0.3 to 0.5 mg to 4 ml of assay system; addition of methylmercuric hydroxide was also made after the addition. Symbols: open circle, myosin; cross, myosin treated first with pyrophosphate; solid line, methylmercuric hydroxide added in the absence of ATP; dashed line, methylmercuric hydroxide added in the presence of ATP; dotted line, methylmercuric hydroxide added in the presence of pyrophosphate.

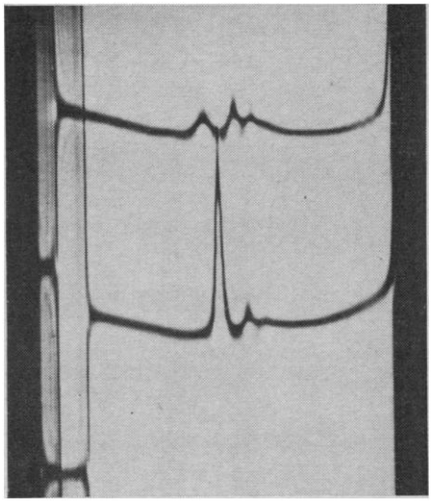

Fig. 2. Ultracentrifuge diagrams of myosin titrated to 5.5 moles of methylmercuric hydroxide per $10^5$ grams of protein. Top, control; bottom, myosin solution made 1 m*M* in pyrophosphate before the addition of methylmercuric hydroxide. Protein concentration 0.5 percent; temperature 20°C; exposure made 67 minutes after reaching 59,780 rev/min, bar angle 50°, sedimentation from left to right.

Ca++-activated adenosine triphosphatase activity occurs, a molecular transformation process also takes place (*5*). In the ultracentrifuge diagram, the sharp peak of myosin is transformed into a faster peak; the adenosine triphosphatase activity is directly proportional to the amount of slow, myosin peak. I proposed (*5*) that binding of mercurial reagent to the strategically situated sulfhydryl group caused a conformational change which reversibly both destroyed adenosine triphosphatase and caused an aggregation. With the same experimental system, I have explored the concept that ATP and pyrophosphate protect myosin not by binding to the active site but by preventing a conformational change.

In the presence of ATP and pyrophosphate, the usual activation of the Ca++-activated adenosine triphosphatase of myosin occurs (Fig. 1) as methylmercuric hydroxide is added until about 3 moles per $10^5$ grams protein is reached; further addition of mercurial produces but slight inhibition. The adenosine triphosphatase activity depends upon its value when the methylmercuric hydroxide was added rather than upon the total amount of the mercurial reagent which is present.

To test the effect of pyrophosphate upon the molecular transformation process, a sample of myosin was titrated to 5.5 moles of methylmercuric hydroxide per $10^5$ grams of protein in the presence and in the absence of 1 m*M* pyrophosphate and examined in both a regular and a wedge cell in the analytical ultracentrifuge. In the control sample lacking pyrophosphate (Fig. 2, top), most of the myosin has been transformed into faster-sedimenting products. In the sample that contains pyrophosphate (Fig. 2, bottom), the sharp myosin peak is largely intact.

Several schemes for explaining these interactions are possible, each based on Koshland's treatment of conformational changes involving active sites (*6*); one such speculation is presented in Fig. 3. Region II is potentially inhibitory (Fig. 3*A*). Upon binding of mercurial reagent to the strategic sulfhydryl group, region II encroaches upon the active site and disrupts it (Fig. 3*B*). Binding of polyanion either imposes restrictions on the conformational change possible for region II or imposes steric hindrance making the sulfhydryl unavailable (Fig. 3*C*). Conceivably this one sulfhydryl group does not exchange with bis-$\beta$-carboxyethyl disulfide (*4*) because the reagent is a polyanion which reacts with the same groups as ATP and pyrophosphate and confers similar protection.

The prevention by ATP of a conformational change in myosin is in agreement with the stabilizing action of ATP on G-actin which has been studied by Katz (*7*). There are also resemblances to more remote systems such as beef kidney membrane, which is protected by ATP against diisopropylphosphorofluoridate inhibition (*8*).

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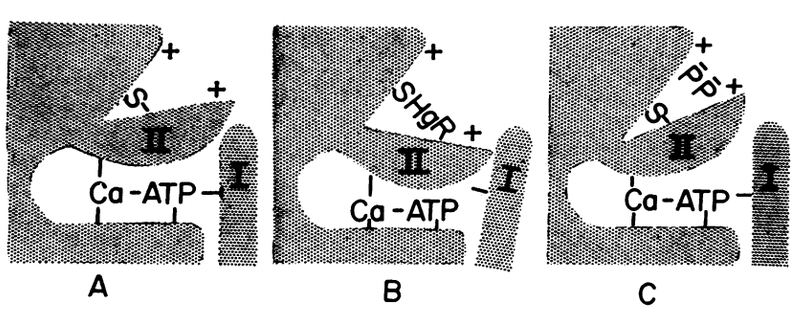


Fig. 3. Possible conformational changes of Ca++-activated adenosine triphosphatase region of myosin.

**References**

1. W. W. Kielley and L. B. Bradley, *J. Biol. Chem.* **218**, 653 (1956).
2. M. F. Morales and K. Hotta, *ibid.* **235**, 1979 (1960).
3. D. Gilmour and M. Gellert, *Arch. Biochem. Biophys.* **93**, 605 (1961).
4. A. Stracher, *J. Biol. Chem.* **239**, 1118 (1964).
5. D. R. Kominz, *Biochim. Biophys. Acta* **51**, 456 (1961).
6. D. E. Koshland, Jr., *Federation Proc.* **23**, 719 (1964).
7. A. M. Katz, *Biochim. Biophys. Acta* **71**, 397 (1963); *Biochemistry* **4**, 987 (1965).
8. L. E. Hokin and A. Yoda, *Biochim. Biophys. Acta* **97**, 594 (1965).

2 August 1965

## Role of Orbital Cortex in Regulation of Thalamocortical Electrical Activity

Abstract. *The orbital region of the cortex in the cat is essential to the occurrence of spontaneous spindle bursts and thalamically induced recruiting responses, observed in both cortex and thalamus. Ablations of the entire dorsal convexity, and of the mesial and cingulate regions of the cortex, failed to interfere with the spindle bursts and recruiting responses, whereas ablations confined to the orbital cortex alone abolished completely these potentials in the cortex and thalamus. Therefore, the orbital cortex appears to be the only region of the neocortex to play a crucial role in the regulation of thalamocortical synchronizing and integrating functions. These functions are believed to be associated with a nonspecific system governing internal inhibition which manifests itself in inattention, drowsiness, and sleep.*

Two kinds of electrocortical activity are known to be dependent upon thalamocortical relationships: (i) spontaneous, spindle-shaped envelopes of 8- to 10-cy/sec waves known as "spindle bursts" (*1*) and (ii) recruiting responses induced by repetitive stimulation of the thalamus (*2*). Thalamocortical interrelationships have been postulated to explain both spindle bursts (*3*) and recruiting responses (*4*).

During cortical ablation experiments to further clarify thalamocortical relationships, it was discovered that ablations of the orbital cortex only could