Actomyosin-Like Protein Isolated from Mammalian Brain

Abstract. *A protein with characteristics similar to actomyosin has been isolated from whole brain of rat and cat. It is soluble in 0.6 molar potassium chloride and insoluble in 0.1 molar potassium chloride. It superprecipitates with magnesium ions and adenosine triphosphate. It has adenosine triphosphatase activity stimulated by either magnesium or calcium ions. Both superprecipitation and adenosine triphosphatase activity are inhibited by* p-*chloromercuribenzoate and Mersalyl but not by ouabain.*

In general, two categories of adenosine triphosphatase have been described based on the cations required for activity. For one group Mg^{2+} or Ca^{2+} is required; for the other, in addition to Mg^{2+}, Na^+ and K^+ are required for maximum enzyme activity. The (Na^+ + K^+)-activated enzyme systems, which function in active transport of Na^+ and K^+ across cell membranes, have been reviewed by Skou (*1*). The biochemical and physiological significance of the Ca^{2+}- or Mg^{2+}-activated enzyme systems are less clear except as related to muscle contraction.

Libet (*2*) in his studies of Ca^{2+}-activated adenosine triphosphatase activity of squid giant axon first suggested that proteins similar to that of the myosin system in muscle may be associated with the conduction of nerve impulses; such proteins would permit permeability changes in the membrane dependent upon structural changes. Bowler and Duncan (*3*) also suggested that contractile adenosine triphosphatases are probably responsible for the control of excitation in nerve and muscle cells. They believe that these enzymes are responsible for the control of passive permeability of excitable cells and possibly of all cells. Germain and Proulx and separately Kadota *et al.* (*4*) described Mg^{2+}- or Ca^{2+}-stimulated activity in isolated synaptic vesicles of rat brain. The former investigators suggested that it functions in the storage and release of acetylcholine.

We now describe the isolation of a Mg^{2+}- or Ca^{2+}-activated adenosine triphosphatase from whole brain of the rat and cat; this enzyme has properties similar to those of muscle actomyosin.

Brain protein was extracted by a modification of the procedure usually used for the extraction of actomyosin from muscle (*5*). Whole brains of rat or cat were cleansed of superficial blood vessels and washed with saline at 0°C. The organs were homogenized with three volumes of 0.6*M* KCl in a bicarbonate buffer at *p*H 8.2 (Weber-Edsall solution) for 30 to 45 minutes at 0°C. The homogenate was kept at 0°C for 16 hours and then centrifuged for 1 hour at 60,000*g*. The clear supernatant extract was diluted with glass-distilled water at 0°C to lower the ionic strength of the KCl to 0.1 mole/liter. The *p*H was brought to 6.3 by the addition of 0.125*M* acetate buffer, *p*H 4.9. After standing for 1 hour the fine precipitate which developed was separated by centrifugation at 12,000*g* for 5 minutes. The precipitate was dissolved in tris-HCl buffer [0.05*M* tris(hydroxymethyl)-aminomethane, 0.6*M* KCl, *p*H adjusted to 7.2 with 0.1*N* HCl] and again precipitated by dilution of the KCl to 0.1 mole/liter with water. After a second reprecipitation the supernatant fluid contained only trace amounts of protein (*6*). The ratio of the readings at 280 nm and 260 nm of the protein dissolved in tris-HCl buffer indicated that approximately 6.5 percent nucleic acids may be present (*7*). The reprecipitated protein constituted approximately 1 to 2 percent of the total brain protein.

Szent-Györgyi (*8*) considers superprecipitation as characteristic of the contractile nature of actomyosin. The protein isolated from brain showed such characteristics (Fig. 1). In 0.1*M* KCl, 10^{-3}*M* tris-HCl buffer (*p*H 7.2), the addition of both 3×10^{-3}*M* $MgSO_4$ and 4×10^{-3}*M* adenosine triphosphate (ATP) caused the protein to form a dense granular precipitate which settled rapidly to the bottom of the tube. Without ATP Mg^{2+} did not have such an effect; ATP alone resulted in some precipitation probably because Mg^{2+} was already present in the protein. The superprecipitation was inhibited by previous incubation of the protein solution at 37°C for 5 minutes with sulfhydryl-blocking agents such as *p*-chloromercuribenzoate (10^{-2} mole/liter) or Mersalyl (10^{-3} mole/liter) (*9*); it was not

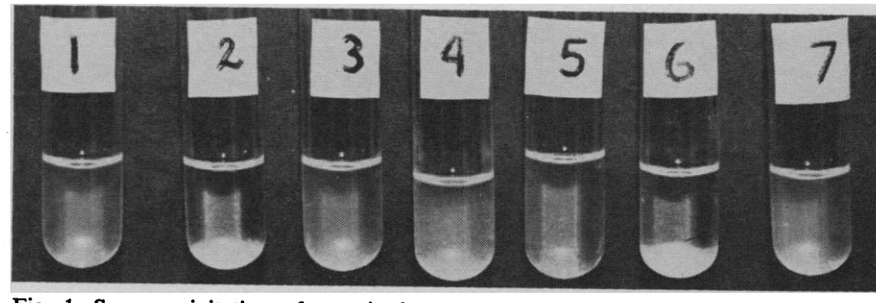

Fig. 1. Superprecipitation of protein isolated from whole brain of the rat and the effect of inhibitors. Each tube contained 0.25 mg of protein in 0.1*M* KCl and tris-HCl buffer, *p*H 7.2 (0.001 mole/liter); (1) no Mg^{2+} or ATP; (2) Mg^{2+} and ATP; (3) Mg^{2+}, no ATP; (4) no Mg^{2+} and ATP; (5) Mg^{2+}, ATP, and Mersalyl (10^{-3} mole/liter); (6) Mg^{2+}, ATP, and ouabain (10^{-4} mole/liter); (7) Mg^{2+}, ATP, and *p*-chloromercuribenzoate (10^{-2} mole/liter). Final concentration of Mg^{2+}, 3×10^{-3} mole/liter; of ATP, 4×10^{-3} mole/liter. Incubated at 37°C for 5 minutes.

Table 1. Effect of ionic concentration on Mg^{2+}- or Ca^{2+}-activated adenosine triphosphatase isolated from whole brain of rat and cat. The assay mixture contained 0.2 mg of protein per milliliter, 5×10^{-4} mole of ATP per liter, and 10^{-3} mole of Mg^{2+}, Ca^{2+}, or both per liter; and 0.05*M* imidazole-HCl buffer [*p*H 6.8 (rat) or *p*H 7.6 (cat)]. Mixtures were incubated at 37°C for 30 minutes. The results are the averages of duplicate determinations of micrograms of P_i released per milligram of protein per 30 minutes. In the absence of Ca^{2+} and Mg^{2+} the values were less than 1 μg of P_i liberated.

Activating ion	KCl (mole/liter)			
	0.03	0.1	0.3	0.6
		Rat		
Mg^{2+}	12.6	12.0	6.2	2.9
Ca^{2+}	8.2	5.1	6.5	6.5
Mg^{2+} + Ca^{2+}	9.6	7.3	6.0	2.6
		Cat		
Mg^{2+}	11.9		16.1	12.1
Ca^{2+}	9.0	16.4	17.7	15.4
Mg^{2+} + Ca^{2+}	9.9	14.2	13.0	8.9

inhibited by ouabain (10^{-4} mole/liter). It was temperature-dependent, occurring much more rapidly at 37°C than at room temperature.

The protein exhibited adenosine triphosphatase activity. The released inorganic phosphate was determined by the Marsh procedure (*10*) adapted for the determination of 0.1 μg of P_i. The *p*H optimum of the activity of the protein isolated from the rat was 6.8; that from the cat was 7.6. This was assayed in a medium containing in final concentration 0.2*M* imidazole-HCl, 0.1*M* KCl, $1 \times 10^{-3}M$ Mg^{2+}, and $5 \times 10^{-4}M$ ATP. The protein hydrolyzed approximately 10^{-2} μmole of ATP per minute per milligram of protein. The enzymatic activity was linear for the first 30 minutes and then gradually diminished over the next 30 minutes. This activity is approximately one-thirtieth that of actomyosin isolated from rabbit striated muscle, half that of smooth muscle (uterus) actomyosin, and twice that of contractile protein from sarcoma cells and blood platelets (*11*).

Table 1 shows the effect of Mg^{2+} and Ca^{2+} on the adenosine triphosphatase activity of the protein in media of different ionic strengths. In the absence of Mg^{2+} or Ca^{2+} the enzymatic activity of the protein was 10 percent of that achieved when $10^{-3}M$ Mg^{2+} was present. In contrast to $(Na^+ + K^+)$-activated adenosine triphosphatase (*1*), the hydrolysis of ATP was almost as good when Ca^{2+} (10^{-3} mole/liter) replaced the Mg^{2+} as the activating cation. Since the addition of both Mg^{2+} and Ca^{2+} were not additive, these ions are very probably stimulating the same enzyme and not two separate enzymes. The enzyme activity of the preparations was dependent upon the ionic strength and the cations of the medium. At low KCl concentration (0.03 mole/liter) Mg^{2+} activation was greatest, and at high KCl concentration (0.6 mole/liter) Ca^{2+} activation was greatest; in the latter case Mg^{2+} had an inhibitory effect. Actomyosin and myosin both have adenosine triphosphatase activity, and both are activated by Ca^{2+}. An important difference is that myosin is inhibited by Mg^{2+} (*12*). At low ionic strength actomyosin predominates, whereas at high ionic strength the addition of ATP causes dissociation of actomyosin and the appearance of myosin adenosine triphosphatase characteristics (*13*). Our data are in general accord with these observations. It is very likely that the preparations contain "actin," "myosin," and "actomyosin." The data suggest that the rat preparation more closely resembles the actomyosin of muscle.

The adenosine triphosphatase activity of the proteins was inhibited in a fashion similar to that observed with superprecipitation. Mersalyl (2.5×10^{-4} mole/liter) reduced the enzyme activity of the protein approximately 80 percent; *p*-chloromercuribenzoate (2.5×10^{-2} mole/liter) reduced the enzyme activity to less than 5 percent. Ouabain in a concentration (10^{-4} mole/liter) effective against the $(Na^+ + K^+)$-activated enzyme (*1*) had only a slight inhibitory effect (approximately 5 percent). Mersalyl has been described as a specific inhibitor of contractile protein adenosine triphosphatase activity (*14*).

The evidence indicates that at least part of the Mg^{2+}- or Ca^{2+}-activated adenosine triphosphatase activity in brain is due to a contractile protein similar to actomyosin.

Contractile proteins in cells may serve a universal function as in cell reproduction (*15*) or a specific function as in striated and smooth muscle contraction (*16*), clot retraction (*11*), or cell movement (*15*). In liver mitochondria it has been described as functioning in the regulation of glycolysis and energy metabolism (*17*). It has been suggested that in nervous tissue it is associated with changes in permeability during excitation (*2*, *3*) and in control of acetylcholine storage and release (*4*). The basis for all its functions is very probably conformational changes transmitted to membranes.

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Cytomembrane Differentiation in the Endoplasmic Reticulum-Golgi Apparatus-Vesicle Complex

Abstract. *Diversity of cytomembrane types is confirmed in hyphae of the fungus* Pythium ultimum *by electron microscopy. A transition in membrane morphology across stacks of dictyosome cisternae (from endoplasmic reticulum-like at one pole to plasma membrane-like at the opposite pole) suggests that dictyosomes of the Golgi apparatus are sites of membrane interconversion.*

Structural and functional diversity among cellular membranes is well established (*1–3*), but still remaining are the questions of how structurally different membrane types are related and whether they are interconvertible. If intermembrane conversion occurs, what is the nature and locus of the transformation? Our electron-microscopic evidence demonstrates a progressive transition in membrane morphology (Figs. 1–7) (*4*) across stacks of dictysome cisternae in hyphae of the plant pathogenic fungus *Pythium ultimum* Trow. These findings are consistent with the interpretation that membrane interconversion occurs in the endoplasmic reticulum-Golgi apparatus-vesicle complex.

Mycelia of *P. ultimum* were cultured at 25°C on potato-dextrose agar (Difco) overlaid with permeable cellophane. Hyphal mats were fixed for