ISOLATION AND PROPERTIES OF AN ACTOMYOSIN-LIKE PROTEIN FROM GLIAL CELLS OF BOVINE BRAIN

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An enriched fraction of glial cells was isolated by centrifugation in a Ficoll—sucrose density gradient from bovine cerebral cortical tissue, and an actomyosin-like protein (AMLP) was obtained from it. The yield of the AMLP was 0.05% relative to glial cell protein. The AMLP was found to contain bound nucleotides and to give reversible association—dissociation reactions characteristic of AMLP under the influence of Mg^{2+} ions and ATP. ATPase of glial AMLP, which is activated by Ca^{2+} ions by a greater degree than Mg^{2+}, differs from the AMLP of neuronal origin.

KEY WORDS: *actomyosin-like protein; glial cells; bovine cerebral cortex; ATPase; dissociation of contractile protein.*

Interest in the study of the location, properties, and functions of the contractile proteins of the brain has increased of late. Contractile proteins of the axoplasm (tubulin in the neurotubules and filarin in the neurofilaments) play a key role in the transport of macromolecules and structures from the body of the neuron along the axon into the region of the nerve endings [12]. The presence of an actin-like protein in presynaptic membranes (PSM) and of a myosin-like protein in synaptic vesicles (SV) can explain the mechanism of exocytosis of mediators during contact between SV and PSM [4, 8, 13]. An actomyosin-like protein (AMLP) has been obtained from isolated nerve endings (synaptosomes) of the animal brain and its properties have been studied [8]

After the discovery of pulsation of astrocytes and oligodendrocytes in tissue culture and contraction of their processes, several workers [6, 10, 13, 18, 20] have suggested that glial cells contain contractile proteins. However, there are no data in the literature on the isolation of such proteins from glia.

The object of this investigation was to isolate an AMLP from an enriched fraction of brain glial cells and to study some of its properties.

EXPERIMENTAL METHOD

The fraction of glial cells was isolated from bovine cerebral cortex preserved in liquid nitrogen by Rose's method [17] with certain modifications [2]. After removal of the blood vessels the brain tissue was washed twice or three times with physiological saline and minced. The minced tissue was suspended in 10% Ficoll (Pharmacia, Sweden) containing 100 mM NaCl and 10 mM Na-phosphate buffer, pH 7.4 (10 ml/g tissue). The suspension was rubbed successively through nylon mesh of 1000, 500, 100, 75, and 50 μ at 0 ± 4°C. The resulting fine suspension was centrifuged in a density gradient (40% sucrose—30% Ficoll suspension in proportions of 1:1.5:2.5 by volume) for 90 min at 53,000g (Spinco L5-65 centrifuge, SW-21 rotor.) Solutions of sucrose or Ficoll also contained NaCl and Na-phosphate buffer. After centrifugation the

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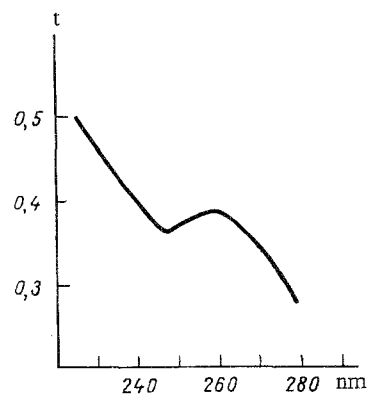

Fig. 1

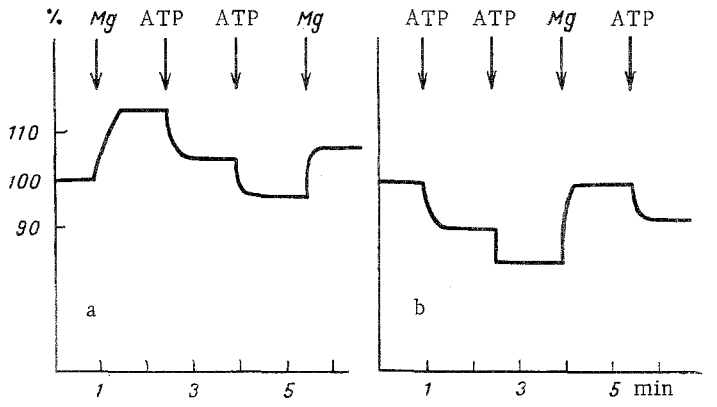

Fig. 2

Fig. 1. Absorption spectrum of AMLP solution. Concentration of AMLP in 0.1 M KCl containing 30 mM Tris-HCl, pH 7.4, was 47 μg/ml (1-cm cell, 20°C). Abscissa, wavelength (in nm); ordinate, optical density (in extinction units).

Fig. 2. Changes in scattering of light by AMLP during association–dissociation reaction. a and b) Different orders of addition of $MgCl_2$ and neutralized solutions of ATP-Na_2 to suspensions of AMLP in 0.125 M KCl containing 30 mM Tris-HCl, pH 7.4. Each arrow denotes microaddition of $MgCl_2$ (with final concentration of 3.1 mM) or of ATP-Na_2 (final concentration 3.4 mM). Quantity of AMLP in sample (0.4 ml) was 40-60 μg. Abscissa, time (in min); ordinate, relative intensity of scattering of light (in %). Typical curves from 6 to 8 experiments are shown.

fraction of glial cells was withdrawn from the upper border of the 30% Ficoll layer, diluted with the same buffered salt solution, and sedimented at 15,000g (30 min). The yield of glia as protein was 10%. The glial cells consisted of astrocytes and oligodendrocytes.

The fraction thus obtained was subjected to osmotic shock followed by freezing and thawing. The AMLP was isolated by Puszkin's method [14-16] with certain modifications [7]. The AMLP was kept in 0.6 M KCl containing 30 mM Tris-HCl, pH 7.6, at 0 ± 4°C for 1-3 days. The yield of AMLP was 0.05% relative to protein of the glial cell fraction.

Protein was determined by Lowry's method [11]. Activity of the ATPases was determined from the accumulation of inorganic phosphate in the course of the reaction (30 min, 37°C) [11]. The incubation medium (1 ml) contained (in mmoles): ATP-Na_2 3; $MgCl_2$ (or $CaCl_2$) 5; Tris-HCl, pH 7.4, 30; KCl 125 or 600; protein 300-400 μg. The aggregation properties of the AMLP were studied by recording the change in the scatter of light at 520 mm (20°C) in a volume of 0.4 ml on the Hitachi-204 (Japan) fluorimeter. The ultraviolet absorption spectrum of the AMLP solution was recorded on an Aminco-Chance (USA) spectrophotometer.

EXPERIMENTAL RESULTS

The content of AMLP (as protein) in the fraction of isolated glial cells of the bovine cerebral cortex was 0.05%. According to data in the literature [7-9, 19] the yield of AMLP from whole mammalian brain may reach 1% as protein. Considering that the properties of AMLP from whole brain and from synaptosomes are practically identical [7-9, 15, 16] this suggests that the AMLP isolated from whole brain is mainly neuronal in origin.

A typical feature of actomyosin and also of brain AMLP, specifically of its actin component, is the presence of bound nucleotides [5, 8]. The absorption spectrum of AMLP from glial cells (Fig. 1) had a maximum of 260 nm, characteristic of nucleotides, indirect evidence in support of the actomyosin-like nature of the isolated protein. Other evidence of the actomyosin-like nature of the isolated protein was its specific reaction to the addition of $MgCl_2$ or ATP to a medium of low ionic strength [7]. Initial addition of Mg^{2+} ions to a suspension of AMLP from glial cells containing 0.125 M KCl and 30 mM Tris-HCl, pH 7.4, caused an increase in the intensity of scattering of light (Fig. 2a), evidence of aggregation and polymerization of the AMLP; the subsequent addition of ATP led to a decrease in the intensity of scattering of light, a posible indication of dissociation of the polymer form of AMLP. If, however, ATP was added first to the suspension of glial cell AMLP in a medium of low ionic

TABLE 1. ATPase Activity of AMLP from Glial Cells (M ± m)

Type of ATPase	Activity, μmoles P_i/mg protein/h	
	0,125 M	0,6 M
Mg-ATPase	2,3±0,5	1,6±0,4
Ca-ATPase	2,5±0,4	1,9±0,5

Legend. Mean values obtained with three preparations of AMLP are given; 2-3 repetitions in each experiment.

strength, a decrease in the intensity of scattering of light was observed, indicating dissociation of the AMLP into actin and myosin components; and the subsequent addition of Mg^{2+} ions to that suspension caused an increase in the scattering of light, indicating the formation of the AMLP complex (Fig. 2b). The reversible association–dissociation reactions of glial cell AMLP can be represented as follows:

$$nAM \overset{Mg^{2+}}{\rightleftarrows} (AM)n \overset{ATP}{\rightleftarrows} nAM \overset{ATP}{\rightleftarrows} nA + nM,$$

$$nAM \overset{ATP}{\rightleftarrows} nA + nM \overset{Mg^{2+}}{\rightleftarrows} nAM \overset{Mg^{2+}}{\rightleftarrows} (AM)_n.$$

Skeletal muscle actomyosin and AMLP of neuronal origin are known to possess ATPase activity stimulated by Mg^{2+} and Ca^{2+} ions; as a rule the Mg-ATPase activity of these proteins in a medium with low ionic strength (0.1 M KCl) exceeds their Ca-ATPase [5, 15]. The AMLP isolated in the present experiments from glial cells possessed ATPase activity. It will be clear from Table 1 that the ratio between the activities of Mg- and Ca-ATPases in the glial preparation differed from that for the AMLP of neuronal origin. Although the differences between the activities of the Ca- and Mg-ATPase of glial AMLP were not statistically significant, in none of the preparations that were isolated did the Mg-ATPase activity exceed the Ca-ATPase activity.

Under conditions of increased functional loading, neurons are known to be surrounded by satellite cells [2, 3]. This compensatory reaction of the glial cells may take place through the increased functional activity of the contractile proteins of these cells.

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