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Interaction of myelin basic protein with the different components of the ATP,Mg-dependent protein phosphatase system

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Myelin basic protein (MBP) reduces the amount of phosphatase activity produced in the kinase F_A-mediated activation of the ATP,Mg-dependent phosphatase. MBP was shown not only to inhibit the activated enzyme, but also to impair the kinase F_A-mediated activation of the inactive phosphatase. In addition MBP prevents the time-dependent inactivation of the catalytic subunit by the modulator protein. These observations point to a regulatory role for MBP in the reversible activation of the ATP,Mg-dependent protein phosphatase by kinase F_A.

Myelin basic protein; ATP; Mg dependence; Protein phosphatase; Protein kinase F_A; Modulator protein

1. INTRODUCTION

All animal tissues examined [1–3] contain the basic components required for the reversible activation of the ATP,Mg-dependent protein phosphatase (F_cM): the 38 kDa catalytic subunit (F_c), which interconverts between the inactive and activated conformation, and the 32 kDa modulator subunit (M), which mediates the activation-inactivation of the catalytic subunit and the protein kinase F_A. The phosphorylation of the modulator by protein kinase F_A is the first step in the activation of the ATP,Mg-dependent phosphatase [4]. Although the mechanism of the in vitro activation of the enzyme has been unravelled down to the molecular level [5–11], little or no information is available at present about the physiological trigger for the in vivo activation or inactivation process. A regulation of the protein kinase F_A activity itself is expected in view of the opposite effects this enzyme can have on the phosphorylation state of glycogen synthase when it either acts as a synthase kinase or as the activating enzyme for a synthase phosphatase [1,12]. The ATP,Mg-dependent protein phosphatase and protein kinase F_A can be used interchangeably between the different sources [1–3]. The ATP,Mg-dependent phosphatase not only has a wide tissue distribution, but it also dephosphorylates an impressive number of protein substrates [13], some of them, like phosphorylase, phosphorylase *b* kinase and glycogen synthase, being key enzymes in the hormonal and metabolic regulation of glycogen metabolism.

Although glycogen metabolism appears to be quantitatively less important in brain than in skeletal muscle, it was recently reported that pig brain has a surprisingly high content of ATP,Mg-dependent phosphatase [2] and kinase F_A [3]. MBP constitutes about 30% of the myelin protein and undergoes a fast phosphorylation turnover in vivo [14]. When phosphorylated either by cyclic AMP-dependent protein kinase or by protein kinase F_A, it is an excellent substrate for the ATP,Mg-

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dependent phosphatase [2]. In addition the phospho- and the dephospho forms of MBP are potent inhibitors of ATP,Mg-dependent phosphorylase phosphatase activity [2].

Here, we have examined the possible role of MBP in the activation-inactivation of the ATP,Mg-dependent protein phosphatase.

2. MATERIALS AND METHODS

Materials and methods were essentially as described in [7,10,11]. The inactive ATP,Mg-dependent phosphatase [10] had a specific activity of 25000 U/mg when measured after a 10 min preincubation at 30°C with kinase F_A, 0.1 mM ATP and 0.5 mM Mg^{2+}, using phosphorylase *a* (2 mg/ml) as substrate. One unit of phosphatase releases 1 nmol [^{32}P]phosphate from ^{32}P-labeled phosphorylase per min at 30°C.

The heat-stable modulator [9], the active catalytic subunit of the ATP,Mg-dependent protein phosphatase [15] and protein kinase F_A [12] were isolated from rabbit skeletal muscle as described. MBP (Eli Lilly, lot P66520) was a generous gift from Dr R.L. Khandelwal (Saskatoon, Canada). Protamine, lysine-rich histones (type V-S) and polylysine (17 kDa) were from Sigma.

To measure the activation of the ATP,Mg-dependent phosphatase or the intrinsic activity of the enzyme in the presence of inhibitory concentrations of MBP or modulator, a trypsin treatment was performed using 50 μg/ml of TPCK-treated trypsin (Sigma), to destroy both heat-stable proteins before the phosphatase assay. The proteolysis (2 min at 30°C) was stopped by the addition of an excess of soybean trypsin inhibitor (Sigma). Trypsin selectively destroys the inactive ATP,Mg-dependent phosphatase, but does not impair the activity of the activated enzyme [7,9,11].

3. RESULTS AND DISCUSSION

3.1. *Effect of MBP on the activation and the activity of the ATP,Mg-dependent protein phosphatase*

Addition of increasing amounts of MBP (not phosphorylated) to the ATP,Mg-dependent phosphatase causes a dose-dependent decrease in the amount of phosphorylase phosphatase activity produced after incubation of the inactive enzyme with protein kinase F_A in the presence of ATP,Mg (fig.1A). The decrease is not a simple inhibition of the phosphorylase phosphatase activity created in the activation step since it cannot be relieved by removal of the inhibitory MBP in a subsequent trypsin treatment (fig.1A). An identical amount of ATP,Mg-dependent phosphatase previously activated by protein kinase F_A is inhibited by similar concentrations of MBP, but this inhibition can be relieved by proteolytic destruction of the inhibitory protein (fig.1B). Activation of the ATP,Mg-dependent phosphatase by protein kinase F_A generally produces more phosphorylase phosphatase activity upon trypsin treatment, and this has been explained by the proteolytic destruction of some of the modulator bound at the inhibitory site on the enzyme [5,9,10]. The trypsinized activated ATP,Mg-dependent protein phosphatase is also completely inhibited by comparable concentrations of non-phosphorylated MBP (fig.1B).

The observation that during activation of the ATP,Mg-dependent protein phosphatase less ac-

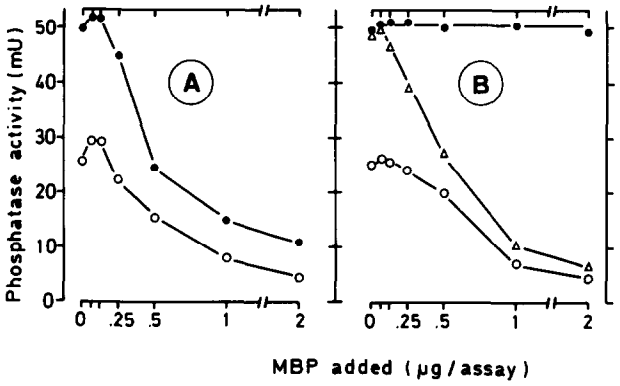

Fig.1. Effect of MBP on the activation and the activity of the ATP,Mg-dependent protein phosphatase. (A) Activation of 150 mU F_cM enzyme by 300 mU kinase F_A in the presence of 0–6 μg MBP in a 30 μl mixture. After preincubation at 30°C for 10 min, a 10 μl aliquot was taken for the determination of the phosphorylase phosphatase activity (30 μl) without (○) or after (●) a trypsin treatment as described in section 2. (B) Inhibition of 50 mU F_cM enzyme, previously activated by 100 mU kinase F_A without (○,●) or with a subsequent trypsin treatment (△), by 0–2 μg MBP per 30 μl assay mixture. The phosphorylase phosphatase activities were determined after (●) or without (○, △) an additional trypsin treatment.

tivity is obtained in the presence of MBP, and that this loss of activity cannot be restored by trypsin treatment (fig.1A) points to the possibility that MBP may interfere in the activation of the phosphatase by protein kinase F_A, thus reducing the amount of active enzyme produced. This idea was confirmed in the experiment shown in fig.2, where increasing amounts of kinase F_A were able to restore the activation of the ATP,Mg-dependent phosphatase in the presence of a concentration of MBP which totally inhibits the potential activity of the enzyme. The amount of active phosphatase could be specifically measured after a subsequent trypsin treatment, which destroyed the MBP, the kinase F_A and the left-over inactive phosphatase as mentioned in section 2. A similar inhibition of the activation of the ATP,Mg-dependent protein phosphatase can be seen by adding purified (free) modulator protein in the activation step (fig.2). The free modulator can also be considered as an alternative substrate for protein kinase F_A.

3.2. *Effect of MBP on the active catalytic subunit of the ATP,Mg-dependent protein phosphatase*

Fig.3A depicts the dose-dependent inhibition of the active catalytic subunit by MBP, and the abolition of this inhibition by subsequent proteolysis. With the substrate phosphorylase *a* at 20 μM in the assay, half-maximal inhibition is seen at about 0.4 μM MBP. This would suggest an enzyme-directed inhibition and not a substrate-directed effect as also mentioned in [2]. In this respect also, MBP is very similar to the free modulator protein, which also inhibits the phosphorylase phosphatase activity at very low (nanomolar) concentrations [16].

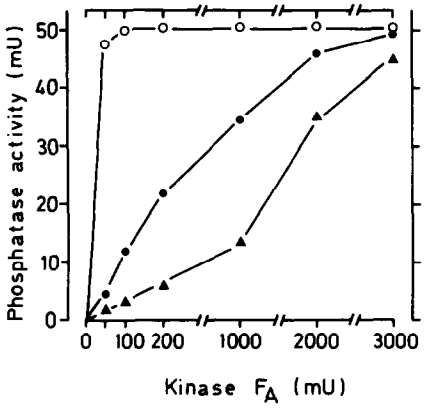

Fig.2. Effect of MBP and 'free modulator' on the kinase F_A-mediated activation of the ATP,Mg-dependent phosphatase. 50 mU F_cM-enzyme were preincubated with (●) or without (○) 4 μg MBP or 50 ng modulator (▲) in the presence of increasing concentrations (0–3000 mU per 30 μl) of kinase F_A, 0.1 mM ATP and 0.5 mM Mg^{2+}. The amount of activated F_cM enzyme was measured by determining the phosphorylase phosphatase activity after a trypsin treatment.

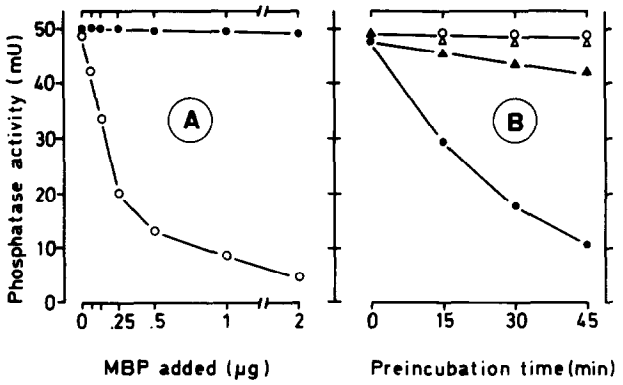

Fig.3. Effect of MBP on the activity and stability of the isolated active phosphatase catalytic subunit. (A) Instantaneous inhibition of the active catalytic subunit by 0–2.0 μg MBP in a 30 μl phosphorylase phosphatase assay without (○) or after (●) a subsequent trypsin treatment. (B) Preincubation at 30°C of 250 mU of the active catalytic subunit with (●, ▲) or without (○, △) 250 ng modulator, in the presence (△, ▲) or absence (○,●) of 20 μg MBP. The total preincubation mixture was 100 μl and at each interval a 20 μl aliquot was taken, treated with trypsin and its phosphorylase phosphatase activity determined as outlined in section 2.

One very specific property of the modulator protein is that it not only inhibits the phosphatase activity but also converts the active catalytic subunit of the ATP,Mg-dependent protein phosphatase to its inactive conformation in a time-dependent manner [1,5,10,17]. This is illustrated in fig.3B, where incubation of the active catalytic subunit with the modulator protein gradually decreased the phosphorylase phosphatase activity, as measured after a trypsin treatment, which destroys the inactive enzyme produced [5]. Incubation of catalytic subunit either alone or in the presence of MBP does not produce any inactive enzyme species, since the phosphorylase phosphatase activity in

both cases remains constant when measured after an identical trypsin treatment. Surprisingly, MBP was able to prevent the inactivation of the active catalytic subunit by the modulator protein (fig.3B). This observation is reminiscent of the stabilizing effect of the deinhibitor protein on the inactivation of the spontaneously active liver protein phosphatase by the modulator [18], but the deinhibitor protein does not interfere in the activation of the ATP,Mg-dependent phosphatase.

The mechanism of stabilization of the active phosphatase conformation by MBP could be explained in several ways. It is not very likely that, due to its very basic nature, MBP complexes itself with that portion of the acidic modulator molecule which binds to its high-affinity site on the catalytic subunit, since addition of MBP after a previous recombination of active catalytic subunit and modulator still prevents the inactivation (not shown). Moreover, other basic proteins such as protamine, lysine-rich histones or synthetically made polylysine do not stabilize the active conformation of the phosphatase. A more likely explanation would be that MBP and modulator compete for the same (high-affinity) binding site on the catalytic subunit which controls the inactivation of the enzyme [5,10]. It is equally possible however that MBP binds at a separate inhibitory site on the catalytic subunit and blocks the inactivation process in an allosteric manner. In either case, MBP or analogous proteins in other tissues may play a stabilizing role in the regulation of the ATP,Mg-dependent protein phosphatase activity.

4. CONCLUSIONS

The possible implication of MBP or MBP-like proteins in the regulation of the ATP,Mg-dependent phosphatase is a very intriguing one. Its abundance in the central and peripheral nervous system as a substrate for the myelin-associated kinase F_A [3] could prevent the activation of the ATP,Mg-dependent multisubstrate protein phosphatase, and thereby increase the phosphorylation level of many proteins in the myelin containing cells. Moreover, it inhibits the active form of this protein phosphatase, while keeping it in the active conformation. MBP does not seem to inhibit its own dephosphorylation [2] so that the activated ATP,Mg-dependent phosphatase may still function as an MBP phosphatase in vivo.

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