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Inhibition of Adenosine Triphosphatase Activity in Brain Microtubule Proteins by S100 Protein

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S100 protein exhibited an inhibitory effect on the adenosine triphosphatase activity of brain microtubule proteins in a concentration-dependent manner. In the presence of Ca^{2+} , maximal inhibition was about 90% (only about 10% of the original activity remained), while the maximum inhibition in the presence of Mg^{2+} was about 30%. Zn^{2+} , which can bind both microtubule proteins and S100 protein, had little effect on the inhibitory action of S100 protein in the presence or absence of Ca^{2+} . The inhibition by S100 protein was reduced by chlorpromazine.

Keywords—S100 protein; microtubule protein; ATPase; chlorpromazine; zinc; calcium

Microtubules have architectural and contractile roles in nearly all eucaryotic cells; for example, they are involved in axonal transport, chromosomal movement and hormonal secretion.¹⁾ The motive force of cell motility depends on the hydrolysis of adenosine 5'-triphosphate (ATP). It has been reported that an ATPase activity is present in brain microtubule proteins.²⁻⁴⁾ The activity is insensitive to several inhibitors of membrane ATPase and remains bound to the microtubule proteins through successive cycles of polymerization and depolymerization.^{3,4)} These results suggest that ATPase in microtubule proteins is associated with the motility of cytoplasm.

S100 protein has been shown to regulate microtubule assembly and disassembly⁵⁻⁷⁾ and the phosphorylation of several proteins.^{8,9)} These regulations are Ca^{2+} dependent. S100 protein also binds to Zn^{2+} with high affinity and induces the inhibition of microtubule assembly.^{6,10-12)} However, the functions of S100 protein have not been established in detail.

We report here that S100 protein inhibits Ca^{2+} -ATPase activity in porcine brain microtubule proteins, and the inhibition by S100 protein is reduced by chlorpromazine.

Materials and Methods

Materials—Diethylaminoethyl(DEAE)-Sephadex A-50, and Sephadex G-75 were purchased from Pharmacia, 2-(*N*-morpholino)ethanesulfonic acid (MES) from Boehringer, Chlorpromazine from Sigma and [γ -³²P]ATP from ICN Radiochemicals. Other chemicals used were of the highest quality commercially available.

Preparation of Proteins—S100 protein and calmodulin were purified from porcine brain by ammonium sulfate fractionation followed by column chromatography on DEAE-Sephadex A-50 and Sephadex G-75.^{11,13)} Microtubule proteins were prepared from porcine brain by three cycles of temperature-dependent assembly and disassembly as described previously.¹⁴⁾

Assay for ATPase—ATPase activity was assayed by measuring the amount of inorganic phosphate released. In the standard assay of ATPase activity, the reaction mixture contained 60 mM MES-KOH (pH 6.5), 2 mM 2-mercaptoethanol, 12.5% (v/v) glycerol, 0.5 mM [γ -³²P]ATP (4—6 $\mu\text{Ci}/\mu\text{mol}$) and 20 μg of microtubule proteins in a final volume of 50 μl . Reactions were initiated by the addition of substrate and stopped by the addition of 20 mM silicotungstic acid in 0.02 N H_2SO_4 after incubation for 30 min at 37 °C. The liberation of inorganic phosphate was

determined with a liquid scintillation spectrometer (Aloka, LSC-651).¹⁵⁾

Protein Concentration—Protein was determined by the method of Lowry *et al.*¹⁶⁾ with bovine serum albumin as a standard.

Results

The effect of S100 protein on the ATPase activity of porcine brain microtubule proteins in the presence of 5 mM Ca^{2+} or 2 mM Mg^{2+} is shown in Fig. 1. Ca^{2+} -ATPase activity was remarkably decreased with increasing concentration of S100 protein. The activity was only about 10% of the original level at over 0.8 mg/ml S100 protein. On the other hand, in the presence of Mg^{2+} the ATPase activity was decreased to a lesser extent by adding S100 protein. The concentrations of S100 protein required for half-maximal inhibition were 0.31 mg/ml in the presence of Ca^{2+} and 0.41 mg/ml in the presence of Mg^{2+} .

Calmodulin is a Ca^{2+} -binding protein which is evolutionarily related to S100 protein and is present in large amounts in the brain.¹⁶⁾ When porcine brain calmodulin (0–0.7 mg/ml) was added to the reaction mixture instead of S100 protein, calmodulin had no effect on the Ca^{2+} -ATPase activity of microtubule proteins (data not shown).

Figure 2 illustrates the dependence of the ATPase activity of microtubule proteins on Ca^{2+} concentration. ATPase activity without S100 protein was slightly stimulated by the addition of Ca^{2+} . When S100 protein (0.45 mg/ml) was present, the ATPase activity decreased with increasing Ca^{2+} concentration, showing a plateau level at over 2 mM Ca^{2+} . These results suggest that high concentrations of Ca^{2+} are required for effective ATPase inhibition by S100 protein.

Zn^{2+} has been shown to bind both S100 protein and microtubule proteins.^{10–12,17)} We therefore examined the effect of Zn^{2+} on the ATPase activity (Table I). In the absence of Ca^{2+} and S100 protein, the ATPase activity was found to be inhibited slightly by adding Zn^{2+} (S100 protein also did not effectively inhibit the ATPase activity in the absence of Ca^{2+}). In the presence of 5 mM Ca^{2+} , addition of Zn^{2+} did inhibit the ATPase activity, but the extent of

Fig. 1. Effect of S100 Protein on the ATPase Activity in Microtubule Proteins

ATPase activity was measured as described in Materials and Methods, in media containing 60 mM MES-KOH (pH 6.5), 2 mM 2-mercaptoethanol, 12.5% glycerol and 0.5 mM $[\gamma\text{-}^{32}\text{P}]\text{ATP}$, made up to contain the indicated concentration of S100 protein. Results each represent the average of three individual experiments. The concentration of microtubule proteins was 0.4 mg/ml. ○, 5 mM CaCl_2 ; ●, 2 mM $\text{Mg}(\text{CH}_3\text{COO})_2$.

Fig. 2. Dependence on Ca^{2+} Concentration of ATPase Activity in the Presence or Absence of S100 Protein

Enzyme activity was measured under the standard conditions modified to the indicated concentrations of Ca^{2+} . The concentrations of microtubule proteins and S100 protein were 0.4 and 0.45 mg/ml (21 μM), respectively. ○, (–) S100 protein; ●, (+) S100 protein.

TABLE I. Effect of Zn^{2+} on ATPase Activity in the Presence or Absence of S100 Protein

Conditions	Zn^{2+} (mM)	(-)S100 nmol/30 min	(+)S100 nmol/30 min	Inhibition ^{a)} (%)
(-)Ca ²⁺	0	1.74	1.24	29
	0.1	1.52	1.28	16
	0.2	1.48	1.26	15
	0.4	1.38	1.30	6
(+)Ca ²⁺	0	3.00	0.74	75
	0.1	1.50	0.56	63
	0.2	1.14	0.30	74
	0.4	1.02	0.22	78

Experimental conditions were the same as for Fig. 1 except that the concentration of S 100 protein was 0.45 mg/ml. a) "Inhibition" is the percent inhibition caused by S100 protein with respect to the ATPase activity under the same conditions except for the absence of S100 protein.

Fig. 3. Effect of Chlorpromazine on Ca²⁺-ATPase Activity in the Presence or Absence of S100 Protein

Experimental conditions were the same as for Fig. 2 except that the concentration of Ca²⁺ was 5 mM and that of chlorpromazine was varied as indicated. ○, (-) S100 protein; ●, (+) S100 protein.

inhibition by S100 protein was high and almost constant, irrespective of Zn^{2+} concentration.

Chlorpromazine reduced the inhibitory effect of S100 protein in a dose-dependent manner under conditions where the ATPase activity was inhibited about 80% by S100 protein (Fig. 3). In the absence of S100 protein, the drug had little effect on the ATPase activity. The inhibition by S100 protein was markedly reduced by over 5 mol of chlorpromazine per mole of S100 protein. This value is about 5 times the molar ratio of chlorpromazine bound to S100 protein reported by Donato.^{18,19)}

Discussion

The present study is the first to find that S100 protein inhibits the ATPase activity contained in brain microtubule proteins. The ATPase activity has been shown to be activated by non-physiologically high concentrations of Ca²⁺.^{15,20)} Potent inhibition by S100 protein was found at higher concentrations of Ca²⁺ (over 1 mM) (Fig. 2), while S100 protein had little effect on the activity at lower Ca²⁺ concentrations. The inhibition of microtubule assembly by S100 protein, on the other hand, was observed when the concentration of Ca²⁺ was 10⁻⁵—10⁻⁴ M.⁵⁻⁷⁾ The binding affinity of S100 protein for Ca²⁺ has been calculated to be 0.1—2 × 10⁻⁴ M.¹⁹⁾ Tubulin, a main component of microtubule proteins, also contains one high-affinity Ca²⁺ site (3.3 × 10⁻⁶ M) and 16 low-affinity sites (2.8 × 10⁻⁴ M).²¹⁾ Thus, the inhibitory effect of S100 protein may due to Ca²⁺ binding to tubulin as well as to S100 protein.

As shown previously,^{6,11,19)} S100 protein in the presence of Zn^{2+} inhibits microtubule

assembly. However, Zn^{2+} has no effect on the inhibition of microtubule ATPase activity by S100 protein (Table I). The molar ratio of S100 protein to tubulin required for half-maximal inhibition is about 5, assuming that the content of tubulin in microtubule proteins is 75% (Fig. 1). On the other hand, we have reported that the molar ratio required to suppress the microtubule assembly is about 2 at 0.2 mM Ca^{2+} .⁷⁾ In addition, Ca^{2+} is one of the most potent inhibitors of microtubule assembly at high concentrations.²²⁾ These results suggest that S100 protein has different effects on ATPase of microtubule proteins from those on the assembly.

It has been reported that chlorpromazine, an antipsychotic drug belonging to the phenothiazine group, binds to S100 protein.^{18,19,23)} S100 protein can interact with phenothiazine-conjugated Sepharose 4B in a Ca^{2+} -dependent fashion and the chlorpromazine binding results in a conformational change of S100 protein. Although the molar ratio of chlorpromazine bound to S100 protein has been reported to be about 1,^{18,19,23)} low concentrations of chlorpromazine showed little effect on ATPase activity in the absence or presence of S100 protein under the conditions employed in the present study (Fig. 3). High concentrations of chlorpromazine were required to reverse the S100 protein-dependent inhibition of ATPase activity, indicating that low-affinity binding sites for chlorpromazine may be present on S100 protein.

Like S100 protein, calmodulin has been demonstrated to modulate the assembly of microtubule proteins in a Ca^{2+} -dependent fashion,^{24,25)} but it has no effect on the ATPase activity. Thus, S100 protein may have a regulatory function on ATPase in microtubules.

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