Effect of S-100 protein on microtubule assembly–disassembly

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S-100 protein was examined for its ability to affect microtubule assembly–disassembly in vitro. Addition of S-100 protein inhibited microtubule assembly in a dose-dependent manner in the presence of Ca^{2+}, but this effect was not observed in the presence of 2 mM EGTA. The interaction of microtubule proteins with S-100 protein was also demonstrated by Ca^{+}-dependent affinity chromatography on S-100 protein–Sepharose. The proteins bound to the Sepharose column comigrated with tubulin as judged by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. These results might suggest a possible physiological role of S-100 protein in the microtubule assembly–disassembly system.

S-100 protein *Microtubule* *Assembly* *Disassembly* *Affinity chromatography* *Ca^{2+}*

1. INTRODUCTION

The brain-specific S-100 protein, first discovered by Moore [1], is a water-soluble, highly acidic protein of M_r 20000 [2,3], and has the characteristic feature of an affinity for calcium [4]. Bovine brain S-100 protein is a mixture of two closely similar components, S-100a and S-100b, both of which have amino acid sequences that are extensively homologous to the sequence for the calcium-binding site of parvalbumin, troponin C and calmodulin [5,6]. However, the physiological role of S-100 protein remains unclear.

We have reported that *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7) coupled to epoxy-activated Sepharose 6B was most useful for the selective purification of S-100 protein (S-100a and S-100b) from soluble extracts of brain [7] and adipose tissue [8]. To clarify the role of S-100 protein, we have examined the interaction of S-100 protein purified by this procedure from brain extracts with microtubule proteins and found that S-100 protein inhibited microtubule assembly in a Ca^{2+}-dependent manner.

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2. MATERIALS AND METHODS

2.1. *Protein preparations*

S-100 protein from bovine brain (mixture of S-100a and S-100b) was purified by Ca^{2+}-dependent affinity chromatography on W-7 epoxy-activated Sepharose as in [7]. Microtubule proteins were prepared from bovine brain in the absence of glycerol by 2 cycles of assembly–disassembly as in [9].

2.2. *Polymerization assay*

Polymerization of microtubule proteins was assayed in a reaction mixture containing in 1.0 ml final vol. 100 mM MES (2-*N*-morpholino)ethanesulfonic acid) buffer (pH 6.4), 0.1 mM $MgSO_4$, 1 mM GTP, 3.2 mg/ml of microtubule proteins, appropriate amounts of S-100 proteins as indicated and Ca^{2+}–EGTA buffer [10]. After a 5 min incubation at 37°C, microtubule polymerization was initiated by addition of GTP and the change in A_{320} with time was recorded [11].

2.3. *Others*

S-100 protein was coupled to CNBr-activated Sepharose 4B (Pharmacia) as in [12]; about 1 mg S-100 protein was linked to 1 ml Sepharose.

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SDS–polyacrylamide gel electrophoresis was done in 0.1% SDS–15% polyacrylamide as in [12].

3. RESULTS AND DISCUSSION

Microtubule assembly in the absence of free Ca^{2+} occurred with a short lag after GTP addition, and turbidity developed in a characteristically sigmoidal fashion (fig. 1). When the [free Ca^{2+}] was raised to 19.7 μM, microtubule assembly occurred with a slightly increased lag time and a reduction in maximum A_{320} of about 5%. At the same [free Ca^{2+}], inclusion of S-100 protein resulted in inhibition of microtubule assembly in a dose-dependent manner (fig.1). However, S-100 protein did not inhibit microtuble assembly in the absence of free Ca^{2+}. These results clearly indicate that S-100 protein inhibits microtubule assembly in a Ca^{2+}-dependent manner. About 1.2 mg S-100 protein/ml produced 50% inhibition of microtubule assembly.

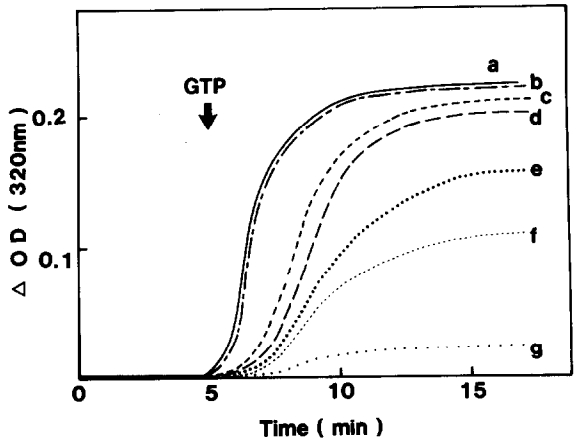

Fig.1. Ca^{2+}-dependent inhibition of microtubule assembly by S-100 protein. The time course of microtubule assembly at 37°C was monitored by light scattering at 320 nm: (a) microtubule proteins + 2 mM EGTA; (b) microtubule proteins + 2 mM EGTA + 2.4 mg S-100 protein/ml; (c) microtubule proteins + 19.7 μM free Ca^{2+}; (d) microtubule proteins + 19.7 μM free Ca^{2+} + 0.3 mg S-100 protein/ml; (e) microtubule proteins + 19.7 μM free Ca^{2+} + 0.6 mg S-100 protein/ml; (f) microtubule proteins + 19.7 μM free Ca^{2+} + 1.2 mg S-100 protein/ml; (g) microtubule proteins + 19.7 μM free Ca^{2+} + 2.4 mg S-100 protein/ml.

The interaction of microtubule proteins with S-100 protein was also demonstrated by Ca^{2+}-dependent affinity chromatography on S-100 protein–Sepharose. Bovine brain (20 g) was homogenized in a glass–teflon homogenizer in 50 ml 25 mM Tris–HCl (pH 7.5), 50 mM NaCl, 2 mM $MgCl_2$, 1 mM EGTA and 1 mM dithiothreitol (buffer A). The homogenate was centrifuged at 105 000 × g, for 60 min. Exogenous $CaCl_2$ was added to the supernatant solution to balance the EGTA and maintain [free Ca^{2+}] at 100 μM. Then the solution was applied to the S-100 protein–Sepharose column (1.2 × 5 cm) equilibrated with buffer B (same composition as buffer A but 100 μM $CaCl_2$ instead of 1 mM EGTA). The column was washed with buffer B until UV absorbance at 280 nm returned to the base-line level. The buffer was changed to buffer A. The typical elution profile of this Ca^{2+}-dependent affinity chromatography of S-100 protein is shown in fig.2. As shown, an EGTA elutable protein peak was observed. Fig.3 shows the SDS–polyacrylamide gel electrophoretic pattern of the S-100 protein–Sepharose binding proteins from bovine brain, stained with Coomassie brilliant blue. The M_r of the major

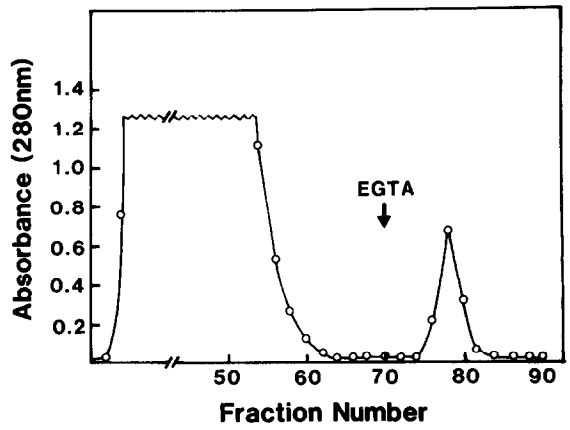

Fig.2. Affinity chromatography on S-100 protein–Sepharose from bovine brain. The soluble fraction from bovine brain was applied to S-100 protein–Sepharose. The column was washed with buffer B containing 100 μM Ca^{2+}. At the point indicated by arrow, the buffer was changed to buffer A containing 1 mM EGTA. Fractions (1 ml) were collected and their absorption at 280 nm was monitored (○—○).

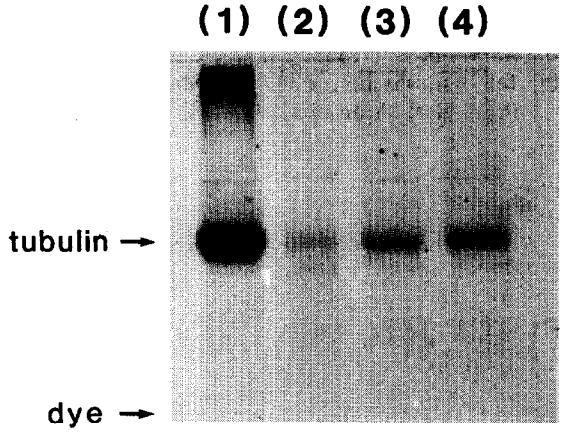

Fig.3. Electrophoretic analysis of isolated proteins from S-100 protein–Sepharose column. Electrophoresis was performed in 0.1% SDS–15% polyacrylamide as in [12]: (1) twice-cycled microtubule protein (10 μg); (2) fraction 76 (5 μl); (3) fraction 78 (5 μl); (4) fraction 80 (5 μl) in fig. 1.

band was about 54000 which comigrated with tubulin dimer. Other minor bands were also observed but the electrophoretic pattern of the S-100 protein–Sepharose binding proteins was very similar to that of twice-cycled microtubular protein, suggesting that S-100 protein interacts with the microtubular protein; i.e., tubulin or microtubule-associated proteins. To test the ability of tubulin dimer to bind to the S-100 protein–Sepharose, 20 mg tubulin dimer purified on a phospho-cellulose column [9] was applied to the column (1.2 × 5 cm) equilibrated with buffer B and eluted with buffer A. However, tubulin dimer did not bind to the column and tubulin dimer was not detected in the EGTA-eluted fraction but was detectable in the flow through.

In 1972, Weisenberg first reported that removal of Ca^{2+} from brain microtubule proteins was necessary for in vitro polymerizaion of microtubules and suggested that Ca^{2+} may play an important role in regulating microtubule assembly [14]. Recently, Ca^{2+} sensitivity of microtubule assembly in vitro has been reported to be modulated by a Ca^{2+}-sensitizing factor [15], and calmodulin has been suggested to have this role [11,16]. Calmodulin reportedly bound tubulin dimer in [16], but in [17] calmodulin bound tau factor, one of the microtubule-associated proteins, Ca^{2+}-dependently [17].

Here, we have shown that S-100 protein also inhibited the assembly of microtubule in a Ca^{2+}-dependent manner. Although it seems that rather high concentrations of S-100 (on a molar basis) are required for inhibition of assembly, if this is related to tubulin [18], these data might suggest that two Ca^{2+}-binding proteins, calmodulin and S-100 proteins, regulate the microtubule assembly–disassembly in a Ca^{2+}-dependent manner. Studies are currently in progress to identify the S-100 protein binding component in microtubular protein. Although tubulin dimer and the several minor proteins could be obtained by affinity chromatography on S-100 protein– Sepharose (fig.3), it is of interest that purified tubulin dimer did not bind to the S-100 protein–Sepharose. Therefore, it is likely that microtubule-associated protein such as tau bind to S-100 as well as to calmodulin [17].

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