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EFFECT OF CALCIUM IONS ON THE INTERACTION OF S-100 PROTEIN WITH MICROTUBULE PROTEINS

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In the presence of Ca^{2+}, S-100 protein suppressed microtubule assembly in vitro in a concentration-dependent fashion and the inhibition was restored by adding EGTA. The binding of microtubule proteins to S-100 protein was examined using affinity chromatography on S-100 protein attached to Sepharose 4B. When tubulin or microtubule-associated proteins (MAPs) were applied to an S-100 protein-Sepharose 4B column, they were bound to the column in the presence of Ca^{2+}, but were not released by adding EGTA. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis indicated that the proteins bound to the column, which were eluted with 0.5 M KCl plus EGTA, consisted of tubulin or tau proteins.

KEYWORDS——S-100 protein; microtubule assembly; tubulin; tau protein; calcium; affinity chromatography

S-100 protein, which is present in large amount in several tissues including cerebrum, cerebellum, brain stem, and adipose tissue, is an acidic Ca^{2+}-binding protein with a molecular weight of 21,000.[1] The amino acid sequences and configurations of Ca^{2+}-binding domains are similar to those of calmodulin, troponin C, and parvalbumin.[2] Recently, S-100 protein has been reported to inhibit brain microtubule assembly in the presence of Ca^{2+},[3] though the precise mechanism of the inhibition is not yet known. Here we report research on the interaction of S-100 protein with the components of microtubule proteins, using S-100 protein-affinity chromatography.

Microtubule proteins were isolated from porcine brain by three cycles of temperature-dependent assembly and disassembly.[4] The separation of tubulin from MAPs was achieved by phosphocellulose chromatography.[5] Heat-stable MAPs were prepared from glycerol-free microtubule proteins as described previously.[6] S-100 protein was purified from porcine brain according to the method of Isobe et al.[7] Tubulin and S-100 protein were electrophoretically pure. The heat-stable MAPs consisted mainly of high-molecular-weight proteins and tau proteins with several minor contaminations.

Microtubule assembly was monitored at 37°C by measuring turbidity change at

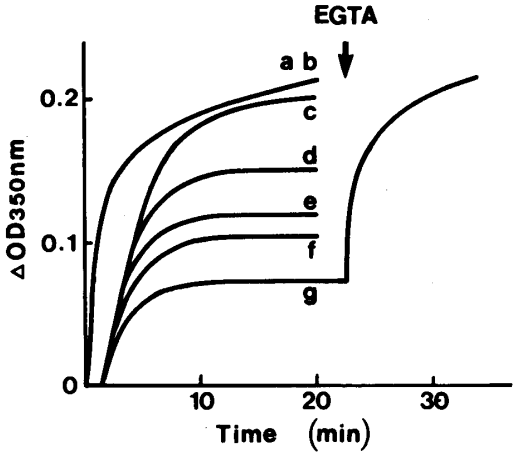

Fig. 1. Inhibition of Microtubule Assembly by S-100 Protein

The concentration of microtubule proteins was 1.5 mg/ml. a, 0.4 mM EGTA; b, 0.4 mM EGTA + 0.45 mg/ml S-100 protein; c, 0.2 mM $CaCl_2$; d, 0.2 mM $CaCl_2$ + 0.16 mg/ml S-100 protein; e, 0.2 mM $CaCl_2$ + 0.29 mg/ml S-100 protein; f, 0.2 mM $CaCl_2$ + 0.45 mg/ml S-100 protein; g, 0.2 mM $CaCl_2$ + 0.61 mg/ml S-100 protein. At the arrow indicated on curve (g), 1 mM EGTA was added to the solution.

350 nm.[6] The reassembly solution consisted of 80 mM 2-(N-morpholino)ethanesulfonic acid (MES)-KOH (pH 6.5), 1 mM $Mg(CH_3COO)_2$, and either 0.2 mM $CaCl_2$ or 0.4 mM EGTA in a final volume of 1 ml. Assembly was initiated by adding GTP and raising the temperature from 4°C to 37°C.

S-100 protein was covalently coupled to Sepharose 4B according to the manufacturer's instructions (Pharmacia Fine Chemicals). Polyacrylamide gel electrophoresis was carried out in 10% gels in the buffer system of Laemmli[8] containing 0.1% SDS. Protein was determined by the method of Lowry et al.[9] with bovine serum albumin as a standard.

The addition of S-100 protein to the reassembly solution containing 1.5 mg/ml microtubule proteins had little effect on microtubule assembly in the absence of Ca^{2+} (Fig. 1). On the other hand, in the presence of 0.2 mM Ca^{2+}, S-100 protein suppressed the extent of microtubule assembly in a dose-dependent manner. The molar ratio of S-100 protein to tubulin dimers required for 50% inhibition was about 2, assuming that the content of tubulin dimers in microtubule proteins was 75%. A rapid disassembly was observed when S-100 protein was added to reconstituted microtubules (data not shown). The inhibitory effect of S-100 protein was reversed by the addition of EGTA, suggesting that the process is reversible depending on the Ca^{2+} concentrations. These results are consistent with the data reported previously that calmodulin and S-100 protein inhibit microtubule assembly and induce disassembly of microtubules in the presence of Ca^{2+}.[3,10,11]

Figure 2 shows the profiles of S-100 protein-Sepharose 4B affinity chromatography and gel electrophoresis patterns in the presence of SDS. The S-100 protein-Sepharose 4B column (1.2 x 10 cm) was equilibrated with buffer A (50 mM MES-KOH, pH 6.5, 0.5 mM $Mg(CH_3COO)_2$, 1 mM $CaCl_2$, 2 mM 2-mercaptoethanol). Heat-stable MAPs or tubulin dimers were applied and washed with buffer A. The material retained on the column was eluted with buffer B (same composition as buffer A except 2.5 mM EGTA instead of 1 mM $CaCl_2$) and buffer B containing 0.5 M KCl. When heat-stable MAPs were applied to an S-100 protein-Sepharose 4B column, about 75% of the protein was not retained (Fig. 2a). Elution with buffer B did not yield any protein peak. Proteins were eluted with buffer B containing 0.5 M KCl. This fraction contained mainly tau proteins, while the unbound fraction consisted of MAP-2 and tubulin. The replacement of heat-stable MAPs by tubulin dimers

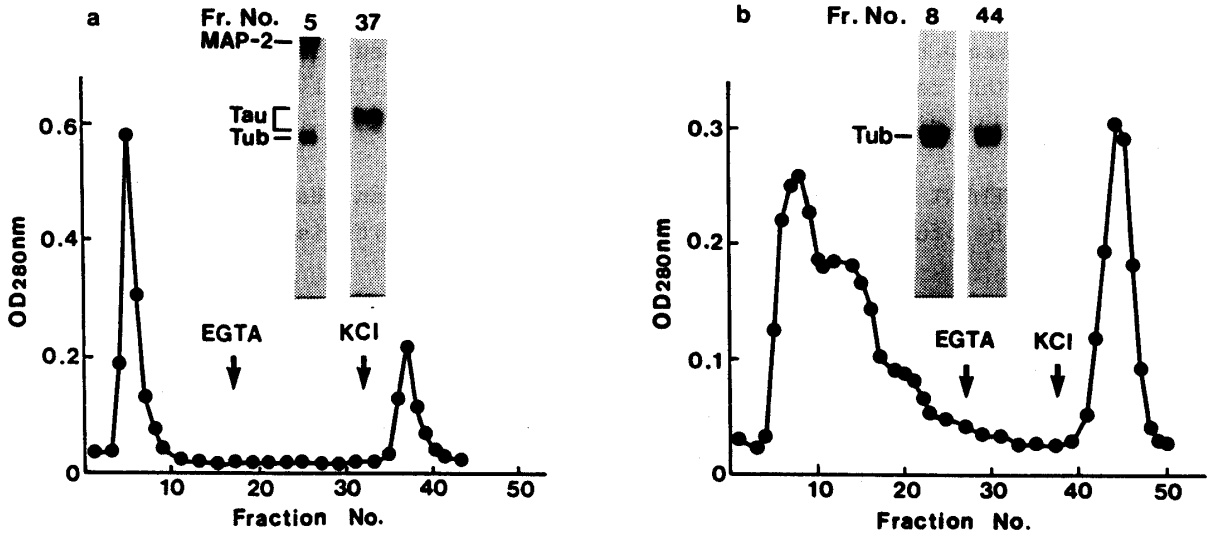

Fig. 2. Profiles of S-100 Protein-Sepharose 4B Affinity Chromatography

Heat-stable MAPs (a) or tubulin dimers (b) were applied to an S-100 protein-Sepharose 4B column. The column was washed with buffer A. At the points indicated by the arrows, the buffer was changed to buffer B (EGTA) or to buffer B containing 0.5 M KCl (KCl). Fractions (1.2 ml) were collected and their absorption at 280 nm was monitored. Unbound and bound fractions were analyzed by SDS-polyacrylamide gel electrophoresis.

caused the increase in the amounts bound to the column and the bound proteins were only eluted with buffer B containing 0.5 M KCl (not with buffer B)(Fig. 2b). There are two discernible trailing shoulders in the unbound fraction that suggest the presence of weak interactions. Both fractions revealed the same mobility on SDS-polyacrylamide gels. The same binding was also observed in the absence of Ca^{2+} (data not shown).

The present results demonstrate that S-100 protein inhibits microtubule assembly in a Ca^{2+}-dependent manner. However, the interaction is not considered to be regulated by Ca^{2+}-concentration, since addition of EGTA did not elute the bound proteins. Calmodulin has been shown to interact with microtubule proteins in a Ca^{2+}-dependent manner.[9,10] Thus S-100 protein and calmodulin were different in their mode of interaction with microtubule proteins. The hydrophobic region of S-100 protein, calmodulin, and troponin C, which is exposed by adding Ca^{2+}, has been shown to be related to the function of these proteins and their interactions with some drugs, with their binding proteins by hydrophobic chromatography, and with fluorescent probes.[12] Recently, Tanaka et al.[13] reported that hydrophobic interactions of these proteins with ω-aminoalkyl-agarose is different among these proteins and that an ionic interaction may also be included. Our results show that the components of microtubule proteins bind to S-100 protein-Sepharose 4B and suggest that an ionic interaction is predominantly responsible for this binding. The binding was found in the absence of Ca^{2+} and was dissociated by increasing ionic strength. Microtubule proteins are known to contain tubulin dimers and MAPs including MAP-2 and tau proteins which promote tubulin assembly

and stabilize reconstituted microtubules.[14] Recent evidence suggests that the tubulin dimer seems to be an S-100 protein binding component in microtubule proteins.[3b] Our results suggest that S-100 protein is likely to interact not only with tubulin dimers but also with tau proteins.

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