Effect of S-100 protein on assembly of brain microtubule proteins in vitro

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S-100 protein inhibits the assembly of brain microtubule proteins in vitro in the presence of 10 μM free Ca^{2+}. The S-100 effect is generally greater on the rate than on the extent of assembly, and even greater as the microtubule protein concentration decreases and the time of preincubation between S-100 and microtubule proteins before GTP addition increases, at a given S-100/tubulin dimer molar ratio. The S-100 effect is greatly enhanced in the presence of physiological concentrations of K^+ and is completely reversed by EGTA.

S-100 protein *Microtubule protein* *Calcium* *Potassium* *Assembly*

1. INTRODUCTION

S-100 [1] is an acidic Ca^{2+}-binding protein [2,3], present in large amounts in the nervous tissue and detectable in significant amounts in well-defined cell types of non-nervous organs [4–9]. S-100 has also been found to be associated with axonemes of the cilia of ependymal cells in the mammalian brain [10], of the epidermal cells of a planarian [11] and of the marine protozoan *Euplotes crassus* (D. Cocchia, S. Ruffioni, F. Michetti and R.D., in preparation), but is absent from the tracheal cilia [11] in accordance with its non-ubiquitous distribution. These observations, together with the finding that S-100 has a high sequence homology with calmodulin and other Ca^{2+}-binding proteins [12,13], prompted a study of the in vitro effects of S-100 on assembly and disassembly of brain microtubule (MT) proteins. The results indicate that S-100 inhibits the assembly of MT proteins in a dose-dependent way in the presence of 10 μM free Ca^{2+}, the effect being greatly enhanced in the presence of 0.12 M K^+, and greatly potentiates the disassembling effect of 0.1–1 mM Ca^{2+}. All the S-100 effects are completely reversed by EGTA.

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Abbreviations: SDS–PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; MT, microtubule; MES, 2-morpholinoethanesulfonic acid; EGTA, ethylene glycol bis(2-aminoethyl-ether)-*N,N',N'*-tetra-acetic acid; A_{350}, absorbance at 350 nm; PC, phosphocellulose

2. MATERIALS AND METHODS

2.1. Purification of S-100 protein

S-100 was purified from ox brain as in [1] with the modifications in [14]. It was ~100% pure by SDS–PAGE and densitometric scanning of the relative gel (not shown). The M_r of S-100 was assumed to be 21000 [15].

2.2. *Purification of MT proteins and assay of MT protein assembly*

MT proteins were obtained from adult Wistar rat brains by 3 cycles of polymerization–depolymerization [16]. SDS–PAGE of the MT protein preparation showed that tubulin was 80–85% pure, as judged by densitometric scanning of the relative

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gel (not shown). Twice-cycled MT proteins were stored at −80°C in 0.1 M MES (Fluka, Buchs) (pH 6.7), 1 mM EGTA, 1 mM $MgCl_2$, 1 mM GTP (Boehringer, Mannheim) and 4 M glycerol. On the day of experiments, the solution was subjected to the third cycle of polymerization–depolymerization. The M_r of the tubulin dimer was assumed to be 110000. Assembly of MT proteins was initiated by adding GTP to a final concentration of 1 mM at 37°C. Assembly was continuously monitored spectrophotometrically with a Beckman 35 spectrophotometer at 37°C as the increase in A_{350}. Details of experiments are given in the figure legends.

2.3. *Other procedures*

The free Ca^{2+} concentration in the presence of EGTA was calculated on the basis of a binding constant of 4.7×10^5 M^{-1} [17]. No attempt was made to measure the free Ca^{2+} concentration in the presence of S-100 and/or GTP. Protein was measured as in [18].

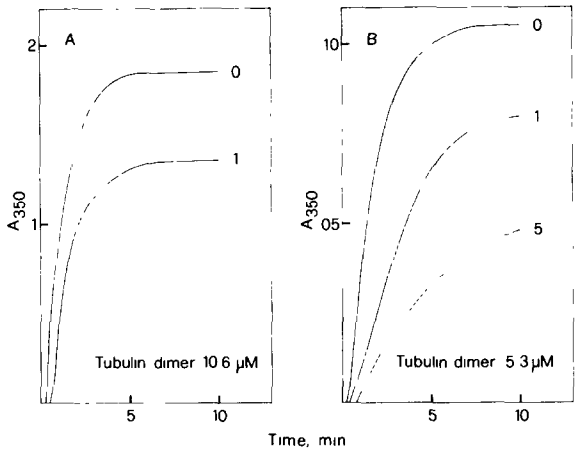

Fig.1. Effect of S-100 on assembly of MT proteins in the absence of K^+: (A) MT proteins corresponding to 10.6 μM tubulin dimer were incubated at 37°C in 0.1 M MES (pH 6.7), 1 mM EGTA, 1 mM Mg^{2+}, 10 μM free Ca^{2+} for 5 min in the absence and in the presence of S-100. At zero time GTP was added; (B) The test was repeated using 5.3 μM tubulin dimer. Figures refer to the S-100/tubulin dimer molar ratio in single tests.

3. RESULTS AND DISCUSSION

In the presence of 10 μM free Ca^{2+} and absence of K^+, preincubation of 10 μM S-100 with 10.6 μM tubulin dimer for 5 min at 37°C before GTP addition results in a significant inhibition of the subsequent assembly of MT proteins as compared to the control situation (fig.1A). Decreasing the tubulin dimer concentration to 5.3 μM results in an enhanced inhibitory effect of S-100, consisting in a significant increase in the lag time between the addition of GTP and the onset of the turbidity increase, and in a decrease in the rate and the extent of assembly (fig.1B).

Including 0.12 M K^+ in the buffer medium containing 10 μM free Ca^{2+} results in a greater inhibitory effect of S-100 on MT protein assembly, at a given S-100/tubulin dimer molar ratio, as compared to tests conducted in the absence of K^+ (fig.2). With 0 min preincubation between S-100 and MT proteins, at a molar ratio of 0.19, S-100 inhibits the assembly by 25 and 13.5%, 2.5 and 10 min after the starting of the reaction, respectively, these values raising to 65 and 48% at a molar ratio of 0.76, and to 90 and 75% at a molar ratio of 2.28 (fig.2A). The effect is greater on the rate than on the extent of assembly. Adding EGTA at the turbidity plateau results in the complete reversal of the S-100 effect (fig.2A), indicating that the protein exerts its effect by potentiating Ca^{2+}. Increasing the time of preincubation of MT proteins at 37°C from 0–5 min before GTP addition results in a slight decrease in both the rate and the extent of assembly (fig.2B) as in [19]. However, including S-100 in the incubation mixture results in a further decrease in both the rate and the extent of assembly, which is proportional to the S-100 concentration and greater than that registered with 0 min of preincubation (fig.2B). Thus, at a S-100/tubulin dimer molar ratio of 0.19, the assembly is 49.5 and 24.5% reduced 2.5 and 10 min after GTP addition, respectively, these values increasing to 100 and 69% at a molar ratio of 0.76. Again, the addition of EGTA at the turbidity plateau completely reverses the S-100 effect (not shown). The addition of Ca^{2+} ± S-100 at the turbidity plateau results in a decrease in A_{350}, which is even greater as the concentration of S-100 in the mixture increases (fig.2B). On the contrary, adding S-100 to MT proteins assembled in the presence of 10 μM free Ca^{2+} produces only a slight, if any, decrease in A_{350} (not shown).

Under any of the above conditions, bovine

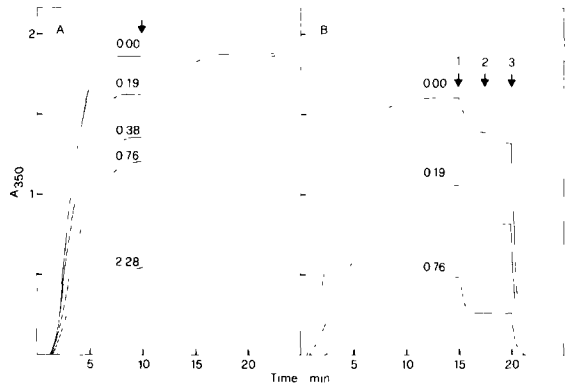

Fig.2. Effect of S-100 on assembly and disassembly of MT proteins in the presence of K^+. (A) MT proteins corresponding to 18 μM tubulin dimer were assembled at 37°C in 20 mM MES (pH 6.7), 1 mM EGTA, 1 mM Mg^{2+}, 0.12 M K^+, 10 μM free Ca^{2+}, 1 mM GTP in the absence and in the presence of increasing S-100 concentrations (no previous preincubation between MT proteins and S-100). After 10 min of reaction EGTA (4 mM final concentration, arrow) was added. Figures refer to the S-100/tubulin dimer molar ratio in single tests. (B) MT proteins corresponding to 18 μM tubulin dimer were preincubated at 37°C in the above buffer without GTP for 5 min in the absence and in the presence of S-100. At zero time GTP was added, and the reaction followed for 15 min at 37°C. Figures refer to the S-100/tubulin dimer molar ratio in single tests. Then, Ca^{2+} (0.1 mM final concentration, arrow 1), S-100 (6 μM final concentration, arrow 2) and Ca^{2+} (1 mM final concentration, arrow 3) were added in this order. MT proteins assembled in the presence of the lowest S-100 concentration tested received 3 μM S-100 after 0.1 mM Ca^{2+}, and MT proteins assembled in the presence of the highest S-100 concentration tested received no further S-100 after 0.1 mM Ca^{2+}.

serum albumin and ovalbumin were without effect on both the assembly and disassembly of MT proteins (not shown).

Calmodulin was the first acidic Ca^{2+}-binding protein shown to affect MT protein assembly in vitro [20,21]. The S-100 effect seems to be comparable to that of calmodulin. In the presence of 10 μM free Ca^{2+} and 0.12 M K^+ (which is the salt normally bathing both S-100 and MT proteins), after 10 min reaction S-100 inhibits the MT protein assembly by 33% at a S-100/tubulin dimer molar ratio of 0.38, by 48% at a molar ratio of 0.76, and by 75% at a molar ratio of 2.28 (fig.2A), while calmodulin inhibits it by 20% at a molar ratio of 0.37, by 35% at a molar ratio of 1.13, and by 75% at a molar ratio of 2.25 [22]. The inhibitory effect of S-100 is even greater as the MT protein concentration decreases at a fixed preincubation time and the time of preincubation before GTP addition increases. This suggests that S-100 interacts in a time-dependent way with one MT protein thereby reducing the ability of tubulin to assemble into supramolecular structures. SDS–PAGE of both the pellet and the supernatant obtained by centrifugation of MT proteins assembled in the absence and in the presence of various S-100 concentrations after 5–15 min preincubation reveals that the MT protein pattern is qualitatively unchanged under any of the above conditions and identical to that obtained with freshly prepared MT proteins, differences concerning exclusively the amount of pelleted material (not shown). This indicates that the increased S-100 effect after preincubation of S-100 with MT proteins in the absence of GTP is not due to changes in the composition of MT proteins because of the preincubation, and is unrelated to the cause(s) responsible for the decrease in the rate and extent of MT protein assembly due to preincubation before GTP addition [19].

Baudier et al. have reported that S-100 potentiates the disassembling effect of mM amounts of Ca^{2+} [23]. Our data show that under physiologic ionic conditions (fig.2) S-100 may have a role in the control of assembly rather than disassembly of MT proteins. The finding that the S-100 effect is enhanced in the presence of K^+ is of interest, as the affinity of S-100 for Ca^{2+} decreases in the presence of 0.1 M K^+ [3]. As also calmodulin is more active on MT protein assembly in the presence than in the absence of K^+ [21], a possible explanation for that finding may be that K^+ renders MT proteins more sensitive to acidic Ca^{2+}-binding proteins.

It remains to be elucidated whether the *a* or the *b* isomer of S-100 [15], or both, is (are) involved in the effect on MT protein assembly, and whether S-100 is also effective in vivo, particularly in cells containing both S-100 and calmodulin. Current data indicate that S-100:

(1) Affects nucleation to a greater extent than elongation of microtubules in vitro;
(2) Inhibits the assembly of phosphocellulose (PC)-purified tubulin; and

(3) Interacts in vitro with PC-tubulin rather than with microtubule-associated proteins (in preparation).

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