Calcium-independent, pH-regulated Effects of S-100 Proteins on Assembly-Disassembly of Brain Microtubule Protein in Vitro*

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At alkaline pH, Ca²⁺ is no longer required for S-100 proteins to inhibit the assembly and to promote the disassembly of brain microtubules in vitro, though the presence of Ca²⁺ significantly favors the S-100 effects. These effects are inversely related to the microtubule protein concentration and directly related to the S-100 concentration and the pH. Ca²⁺-independent, pH-regulated inhibition of assembly of phosphocellulose-purified tubulin by S-100 is also described. The microtubule disassembling effect of S-100 is additive to that of alkali (used to raise the pH), and S-100 further disassembles microtubules after alkalinization. Thus the larger inhibitory effect of S-100 on microtubule assembly at alkaline versus acid pH depends on both a decrease in the assembly rate and an increase in the disassembly rate. Together with previous data on this topic, the present findings indicate that S-100 proteins act on microtubule protein in vitro primarily by binding to tubulin, this event being Ca²⁺-regulated at a given pH, and pH-regulated at a given free Ca²⁺ concentration.

Microtubules (MTs) are cytoskeletal constituents involved in a number of cell functions or events (1). They rapidly assemble and disassemble depending on the functional needs of the cell. The state of assembly of cytoplasmic MTs, which are composed of tubulin plus a number of accessory proteins termed microtubule-associated proteins (MAPs) (2–4), seems to be regulated by the tubulin concentration itself, MAPs, GTP, ATP, Mg²⁺, Ca²⁺, and the pH (2–9). Ca²⁺ inhibits the assembly and promotes the disassembly (8). However, at the concentration present in the cell (0.1 to 10 μM) Ca²⁺ seems not to be capable of affecting the state of assembly of MTs (10). Cytoplasmic factors have been postulated to increase the Ca²⁺ sensitivity of microtubule protein (MTP) (10). The intracellular Ca²⁺ receptor, calmodulin, was identified as one of these factors (11).

Another factor involved in the regulation of the state of assembly of MTs is the pH. At alkaline pH, MTP assemblies at a reduced rate and disassembles at a faster rate (9). There is evidence indicating that changes in the intracellular pH may play a role in the system regulating the state of assembly of MTs (9, 12).

S-100 is a protein fraction originally purified from bovine brain, comprising three small (Mr, 21,000), acidic (pH 4.3), Ca²⁺-binding isoforms, which are structurally related to calmodulin and other Ca²⁺-binding proteins and are modified in their secondary structure by Ca²⁺ to nearly the same extent (for a recent review see Ref. 13). Thus, the S-100 functions are expected to be mediated by Ca²⁺.

Bovine brain S-100, which contains all three isoforms (S-100a, S-100a, and S-100b) and rat brain S-100, which is ≥ 95% S-100b (14), were recently shown to be involved in the control of the assembly-disassembly of brain MTP and to inhibit the assembly of purified tubulin in a Ca²⁺-mediated way in vitro (15–19). Individual S-100 isoforms appear to exert equipotent effects on MTP and purified tubulin assembly-disassembly (19). Turbidimetric and ultrastructural studies have shown that S-100 interferes with both the nucleation and the elongation of MTs in the presence of Ca²⁺ (18, 20) by binding to tubulin (21). Also, some competition between S-100 and MAPs for binding to tubulin has been reported (21). Finally, S-100 proteins have been shown to increase the Ca²⁺ sensitivity of MTP, this effect being dependent on pH and on the KCl concentration (22).

To obtain more detailed information on the role of pH in S-100 effects on MTP assembly-disassembly, we studied the assembly-disassembly of MTP and of purified tubulin in the absence and in the presence of S-100 at various pH values, in the presence and in the absence of Ca²⁺. Data obtained in the presence of Ca²⁺ are consistent with the idea that S-100 increasingly interferes with the nucleation and the elongation of MTs in vitro as the pH rises, at a given S-100/tubulin molar ratio (23). This is most evident at pH 7.5, a condition where the MT number concentration and the mean MT length decrease in the presence of S-100 (23). The data obtained in the absence of Ca²⁺ are presented here. We show that, at the pH rises, Ca²⁺ is no longer required for S-100 to inhibit the assembly of either MTP or purified tubulin, and to promote the disassembly of steady state MTs.

MATERIALS AND METHODS

Purification of Protein—S-100 was purified from bovine and rat brain as described (18). Bovine and rat S-100 proteins were checked for purity by polyacrylamide gel electrophoresis (PAGE) both in the presence (Fig. 1, lanes A and B) and in the absence (Fig. 1, lanes C–E) of sodium dodecyl sulfate (SDS). The S-100 fraction obtained from bovine brain was a mixture of S-100a and S-100b (Fig. 1), which was fractionated no further. Its concentration was calculated by UV spectroscopy using the value of $E_{280}^\text{\text{m}} = 0.344$ (24) since the mixture used here contained nearly equal amounts of S-100a and S-100b by densitometric scanning of the gel (not shown). Rat brain S-100 was ~100% S-100b (Fig. 1). Its concentration was calculated using the value of $E_{280}^\text{\text{m}} = 0.185$ (14). MTP was purified from adult rat brain by three cycles of assembly-disassembly at pH 6.7 (25). Tubulin was separated from MAPs by phosphocellulose chromatography (2) of

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The abbreviations used are: MTs, microtubules; MAPs, microtubule-associated proteins; MTP, microtubule protein; $A_{280}$, absorbance at 350 nm; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; PC-tubulin, phosphocellulose-purified tubulin; EGTa, ethylenebis(oxyethylenenitrilo)tetracetic acid; Mes, 2-(N-morpholino)ethanesulfonic acid.
thrice-cycled MTP. The concentration of MTP and phosphocellulose-purified tubulin (PC-tubulin) was measured by the method of Lowry et al. (26). SDS-PAGE revealed that tubulin represented ~80% of MTP (Fig. 1, lane F) by densitometric scanning of the gel (not shown), that PC-tubulin was essentially pure (Fig. 1, lane G), and that MAPs consisted of high molecular weight polypeptides and of a minor percentage of low molecular weight polypeptides (Fig. 1, lane H). The tubulin dimer concentration of MTP preparations was calculated on the assumption that 1 mg of MTP contained 0.8 mg of tubulin, based on data in Fig. 1, lane F.

**Assay of MTP Assembly-Disassembly—**MTP assembly-disassembly and PC-tubulin assembly were continuously monitored spectrophotometrically at 37 °C as the change in absorbance at 350 nm (A350) (5). Details are given in the legends to figures and tables.

**Other Procedures—**Electrophoretic analyses were performed by the method of Laemmli (27) in the presence of SDS, and by the method of Isobe et al. (28) in the absence of SDS.

**RESULTS**

*Effect of S-100 on MTP Assembly at Various pH Values in the Absence of Ca**2**+—**MTP assemblies in the absence of Ca**2**+ (1 mM EGTA) with the same lag phase but at an ever slower rate and to an ever smaller extent as the pH rises from 6.7 to 7.5 (Fig. 2), in accordance with previous data (9). In the presence of S-100 (either bovine or rat brain), used at a molar ratio of 2.0 with respect to the tubulin dimer, a small but significant increase in the lag phase and a decrease in both the rate and the extent of assembly are registered at pH 6.7 (Fig. 2 and Table I). As the pH rises to 7.1 and 7.5, the efficacy of S-100 as an inhibitor of MTP assembly increases linearly with the pH (Fig. 2 and Table I). When Ca**2**+ to 1 mM (free Ca**2**+) is added to MTP assembled to steady state, MTPs formed in the presence of S-100 disassemble at a slower rate but to a larger extent than those formed in its absence at all pH values tested (Fig. 2). A comparative analysis of S-100 effects on MTP assembly in the absence and presence of Ca**2**+ is presented in Table I. Ca**2**+ mostly favors the S-100 effect at pH 6.7. As the pH rises, Ca**2**+ is no longer a prerequisite for S-100 to increase the lag phase and to decrease the rate and the extent of assembly, though its presence significantly potentiates the S-100 effects.

**Inverse Relationship between the MTP Concentration and the S-100 Effect on Assembly at Various pH Values in the Absence of Ca**2**+—**Experiments performed at various MTP concentrations, keeping the S-100/tubulin dimer molar ratio constant, show that at any pH the inhibitory effect of S-100 is inversely related to the MTP concentration (Fig. 3A) and that the critical concentration for MTP assembly in the presence of S-100 increases, under these conditions, from 3.5 μM tubulin dimer to 4.5 μM at pH 6.7, from 5.5 to 9.0 μM at pH 7.1, and from 7.5 to 13.0 μM at pH 7.5.

**Dose-dependent Effect of S-100 on MTP Assembly at Various pH Values in the Absence of Ca**2**+—**MTPs at a constant concentration were assembled at various pH values in the presence of increasing concentrations of S-100. The plot of the value of A350 at steady state versus the S-100/tubulin dimer molar ratio (Fig. 3B) indicates that the S-100 effect is

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**FIG. 1. SDS-PAGE (12.5% polyacrylamide) of bovine (A) and rat (B) S-100.** Shown is PAGE in the absence of SDS of bovine S-100 (C), rat S-100 (D), and a mixture of bovine and rat S-100 (E) (20% polyacrylamide) and SDS-PAGE (12.5% polyacrylamide) of MTP (F), PC-tubulin (G), and MAPs (H).

**FIG. 2. Ca**2**+-independent effect of S-100 on MTP assembly.** MTP corresponding to 15.15 μM tubulin dimer was incubated at 37 °C in 0.5 ml of 0.1 M Mes, 1 mM EGTA, 1 mM MgCl2 at pH 6.7 (traces a and a'), 7.1 (traces b and b'), and 7.5 (traces c and c') in the absence (traces a, b, and c) and presence (traces a', b', and c') of 30 μM S-100. After 2.5 min (zero time), GTP was added to 1 mM. At steady state, Ca**2**+ was added to 2 mM (~1 mM free Ca**2**+). The rate of assembly and of disassembly was calculated on the steepest portion of curves as ΔA350/min.

**TABLE I**

| Conditions | Lag phase | A350μM | ΔA350/min |
|------------|-----------|--------|-----------|
| pH 6.7     | 30        | 0.258  | 0.240     |
| pH 6.7, S-100 | 40    | 0.220  | 0.129     |
| pH 6.7, Ca**2**+ | 30   | 0.252  | 0.237     |
| pH 6.7, Ca**2**+, S-100 | 30  | 0.115  | 0.032     |
| pH 7.1     | 30        | 0.207  | 0.170     |
| pH 7.1, S-100 | 60   | 0.122  | 0.025     |
| pH 7.1, Ca**2**+ | 30   | 0.205  | 0.165     |
| pH 7.1, Ca**2**+, S-100 | 80  | 0.050  | 0.016     |
| pH 7.5     | 30        | 0.160  | 0.086     |
| pH 7.5, S-100 | 120  | 0.040  | 0.005     |
| pH 7.5, Ca**2**+ | 30   | 0.160  | 0.084     |
| pH 7.5, Ca**2**+, S-100 | ND  | 0.000  | 0.000     |

* Extent of assembly at steady state.
  Rate of assembly calculated on the steepest portion of the appropriate curve of assembly.
  No assembly was registered under these conditions during the time period investigated (20 min).
FIG. 3. Effect of S-100 on MTP assembly at various pH values in the absence of Ca"++. A, dependence of the S-100 effect on MTP concentration. MTP corresponding to the tubulin concentrations indicated was assembled in the absence of Ca"+ (1 mM EGTA) to steady state at pH 6.7 (C and ●), 7.1 (Δ and △), and 7.5 ([□] and ■) in the absence (open symbols) and presence (closed symbols) of S-100 at a fixed S-100/tubulin dimer molar ratio of 2. Results are expressed as the A500 value at steady state versus the tubulin dimer concentration. B, dependence of the S-100 effect on S-100 concentration. MTP corresponding to 15.15 μM tubulin dimer was assembled to steady state as in A at pH 6.7 (○), 7.1 (△), and 7.5 (□) in the presence of increasing concentrations of S-100. Results are expressed as the A500 value at steady state versus the S-100/tubulin dimer molar ratio. C, effect of S-100 on the elongation of MTs. MTP corresponding to 12.15 μM tubulin dimer was incubated in the absence of Ca"+ (1 mM EGTA) at pH 6.7, 7.1, and 7.5 in the absence (○) and presence (□) of 24 μM S-100. After 2.5 min, 20 μl of a suspension of 17.7 mg of MTP/ml, previously assembled to steady state at pH 6.7, 7.1, and 7.5, respectively, and passed five times through a hypodermic syringe (1 ml, 25-gauge needle) to produce MT fragments, were added along with GTP. MT fragments served as nuclei for subsequent addition of MTP, i.e., elongation of MTs. The assembly was followed for 15 min. Results are expressed as the A500 value at steady state versus the pH. In the present experiments, the tubulin dimer concentration increased by 20% after addition of fragments.

FIG. 4. Ca"+-independent effect of S-100 on PC-tubulin assembly. PC-tubulin (15.3 μM) was incubated at 37°C in 0.5 ml of 20 mM Hes, 0.12 M KCl, 1 mM EGTA, 1 mM MgCl2, 1 mM GTP at pH 6.7 (traces a and a') and 7.5 (traces b and b') in the absence (traces a and b) and presence (traces a' and b') of 30 μM S-100. After 2.5 min (zero time), dimethyl sulfoxide was added to 10% (v/v) to initiate the assembly.

The assembly.

Effect of S-100 on Assembly of MT Fragments at Various pH Values in the Absence of Ca"++. The value of A500 at steady state is a measure of the MT mass, i.e., of the MT number concentration times the average MT length (29, 30). In the absence of Ca"++, S-100 reduces the rate (not shown) and the extent (Fig. 3C) of the assembly of MTP supplemented with MT fragments. The effect is small at pH 6.7 and increases linearly with the pH. Practically, no lag phase is registered in these experiments, irrespective of the pH and of the absence or presence of S-100 (not shown).

Effect of S-100 on Assembly of MT Fragments at Various pH Values in the Absence of Ca"++. S-100 from either bovine or rat brain also inhibits the assembly of PC-tubulin in a Ca"+-independent, pH-regulated way (Fig. 4). As observed with whole MTP, the inhibitory effect of S-100 on PC-tubulin assembly is small at pH 6.7 and most evident at pH 7.5, at a fixed S-100/tubulin dimer molar ratio, and, at the pH values tested, the effect is more intense on the initial rate than on the extent of assembly.

S-100- and Alkali-induced Disassembly of MTs at Various pH Values in the Absence of Ca"++. S-100, added to steady state MTs at various pH values in the absence of Ca"++, produces an ever larger disassembly at an ever higher rate as the pH at which assembly was carried out rises (Table II).

Table II

| pH   | A500% | A500/min% |
|------|-------|-----------|
| 6.7  | 7.6   | 12.4      |
| 7.1  | 22.2  | 29.7      |
| 7.5  | 37.5  | 46.8      |

* Extent of assembly expressed as the percent decrease in A500 at the new steady state (10 min after the addition of S-100).

Table III

Combined effect of S-100 and alkali on MTP assembled at various pH values in the absence of Ca"++

MTP corresponding to 18.23 μM tubulin dimer was assembled to steady state in the absence of Ca"++ (1 mM EGTA) at the pH values indicated, after which either NaOH (from a concentrated solution to raise the pH to 7.5) or NaOH plus S-100 (to a final concentration of 20 μM) was added in small volumes. Changes in A500 were followed for 10 min after additions, i.e. until a new steady state was attained. In separate experiments, we standardized the minimal volume of the concentrated solution of NaOH to be added to microtubule suspensions in order to obtain the desired rise in pH.

| pH   | A500% | A500/min% |
|------|-------|-----------|
| 6.7, NaOH | 35.2 | 57.4 |
| 6.7, NaOH-S-100 | 38.1 | 59.3 |
| 7.1, NaOH | 20.3 | 17.3 |
| 7.1, NaOH-S-100 | 28.4 | 34.1 |

* Extent of assembly expressed as the percent decrease in A500 at the new steady state after additions.

* Disassembly rate calculated on the steepest portion of disassembly curves and expressed as the percent decrease in A500/min.
When added along with NaOH (used to raise the pH) to steady state MTs (Table III), S-100 potentiates the effect of alkali, particularly if MTs were assembled at alkaline pH (7.1). Again, S-100 has a larger disassembling effect on MTs assembled at alkaline (7.1) than at acidic (6.7) pH, in agreement with data in Table II. Moreover, S-100 further reduces the $A_{350}$ value when it is added to a MT suspension after alkalization (not shown). Thus, the strong inhibitory effect of S-100 on the extent of assembly at alkaline pH seems to depend on both a decrease in the assembly rate and on an increase in the disassembly rate.

**DISCUSSION**

The results presented in this paper show that S-100 from either bovine brain (S-100a plus S-100b) or rat brain (S-100b) inhibits the assembly and promotes the disassembly of whole MTP and PC-tubulin in vitro in a Ca$^{2+}$-independent, pH-regulated way. S-100 increases the lag phase and decreases the rate and the extent of assembly pH dependently. These observations, together with the progressive increase in the critical concentration for MTP assembly, indicate that S-100 increasingly interferes with the nucleation as the pH rises, in analogy with what occurs under the same conditions in the presence of Ca$^{2+}$ (18, 23). Since the addition of preformed nuclei of MTs to a MTP solution does not abolish the S-100-dependent inhibition of assembly under these conditions, it is conceivable that S-100 may also interfere with the elongation reaction in a pH-regulated way. On the other hand, data in Tables II and III suggest that the S-100 effect on the assembly of MTP supplemented with MT fragments at alkaline pH may not be entirely due to interference with the addition of tubulin dimers onto the growing MT polymers, but may also depend on S-100-induced disassembly of added fragments. Yet, adding S-100 to MTP previously assembled to steady state at various pH values and then sheared to increase the MT number concentration, while keeping the MT mass constant, results in the same disassembly as observed with undisturbed MTs (not shown), and diluting a suspension of MTs 10 times at various pH values produces identical disassembly curves in the absence and in the presence of S-100 (22). Thus, S-100-induced disassembly of added fragments does not contribute to data presented in Fig. 3C to a significant extent.

The extent of MTP assembly, measured as the $A_{350}$ value at steady state, is the net result of the rate at which MTP assemblies and of the rate at which MTs disassemble. Data in Tables II and III show that the MT disassembling effect of S-100 increases with the pH. This explains why experimental points reported in Fig. 3B fall on lines rather than on curves, at least in the S-100 concentration range tested.

The present data further stress the fact that S-100 acts on tubulin (18, 21, 31). Quantitative analyses indicate that the stoichiometry of S-100 binding to PC-tubulin increases with increasing free Ca$^{2+}$ concentrations at pH 6.7, and with the pH (21). Also, at alkaline pH (7.4), Ca$^{2+}$ is no longer required for S-100 binding to PC-tubulin (21). This Ca$^{2+}$-independent binding of S-100 to tubulin at alkaline pH explains the results presented here.

On the contrary, calmodulin has no effect on assembly of purified tubulin (32) and acts on MTP by binding to $\tau$ and MAP2 (32, 33). Of course, the present experiments do not exclude the possibility that S-100 may also interfere with the activity of MAPs. However, data presented elsewhere indicate that S-100-tubulin complexes can be recovered after gel chromatography (Sephadex G-200) and after precipitation with ammonium sulfate, while the same does not occur when S-100 is reacted with MAPs (21). This indicates that the affinity of S-100 for tubulin is higher than that for MAPs. Recently, it has been reported that tubulin and, to a lesser extent, $\tau$ factors bind to Sepharose-immobilized S-100 (34). Binding of S-100 to $\tau$ factors in the turbidity experiments presented here might play a role in the overall effect of S-100 on assembly-disassembly of whole MTP. However, the observation that S-100 inhibits the assembly of PC-tubulin indicates that binding to $\tau$ and to other MAPs is not essential for S-100 to affect the MT assembly-disassembly. Also, in view of the fact that S-100 is not present in axons (Ref. 12) and $\tau$ factors appear to be confined to axons (see Ref. 35 and references cited therein), the interaction between S-100 and $\tau$ factors appears of questionable biological significance.

In spite of the structural relatedness of S-100 to calmodulin and other Ca$^{2+}$-binding proteins (36, 37), the affinity of S-100 for Ca$^{2+}$ is not high, particularly in the presence of physiologic K$^+$ concentrations, with K$^+$ antagonizing the binding of Ca$^{2+}$ to high affinity sites on S-100 (38). Within the cell, S-100 is thus expected not to be capable of amplifying the Ca$^{2+}$ signal. Yet, S-100 affects the MTP assembly both in the absence and in the presence of K$^+$ (18, 22). The increased lability of MTs in the presence of K$^+$ (39) cannot explain this effect of S-100 completely (22). In addition, since $\mu$M Ca$^{2+}$ levels do not saturate the Ca$^{2+}$-binding sites on S-100 (38), one is forced to postulate a Ca$^{2+}$-independent ability of S-100 to affect the MTP assembly, though Ca$^{2+}$ significantly increases the inhibitory effect of S-100 (Table I). Precedent exists for Ca$^{2+}$-independent association of S-100, as well as of calmodulin, with target proteins (40-47). Particularly, data have been presented suggestive of Ca$^{2+}$-independent association of calmodulin with MTs (44).

At alkaline pH, the tubulin-tubulin interactions are expected to be reduced since the tubulin molecules become more negatively charged. This is in line with the decrease in the ability of purified tubulin to polymerize at alkaline pH (48) (see also Fig. 4) and with the observation that the enzymatic cleavage of a small fragment from the C-terminal end of both $\alpha$ and $\beta$ subunits of tubulin, which is negatively charged, results in an increase in the ability of purified tubulin to polymerize (49, 50). It is not clear at present how the rise in pH, which also induces an increase in the net negative charge of S-100, results in the potentiation of the S-100 inhibitory effect on MTP and PC-tubulin assembly. MAPs were reported to coassemble with tubulin at a constant stoichiometry irrespective of pH (9), and S-100 seems to interact with tubulin in a pH-dependent way (21, 31), whereas some uncertainty still remains on S-100 binding to MAPs (21, 34). The critical concentration for MTP assembly increases dramatically with the pH in the presence of S-100 and absence of Ca$^{2+}$ (Fig. 3A), indicating that an increasingly larger proportion of MTP does not take part in the formation of MTs. Since the ability of S-100 to disassemble steady state MTs also increases with the pH, one can tentatively conclude that the steady state tubulin flux or treadmilling is increased by the presence of S-100. In this respect, since the treadmilling increases with increasing pH (9), the effect of S-100 seems to add to that of pH. Accordingly, while barely detectable differences in the MT number concentration and in the mean MT length are observed at pH 6.7 in the presence of S-100, a decrease in both parameters is registered at pH 7.5 in the presence of S-100 (not shown). Similar experiments performed in the presence of 10 $\mu$M free Ca$^{2+}$ gave qualitatively similar results at pH 7.5, while, due to the presence of Ca$^{2+}$, a significant decrease in the MT number concentration and an increase in the mean MT length were registered at pH 6.7 (23). The latter
finding is due to the small MT disassembly effect of S-100 and to the larger effect of S-100 on the nucleation than on the elongation at pH 6.7 (20, 23).

On the basis of kinetic data and on counts of MTs formed in the absence and presence of S-100 we proposed that S-100 interferes with both the nucleation and the elongation of MTs by sequestering unassembled tubulin (18, 23). The effect on elongation is most evident at pH 7.5, both in the presence (23) and in the absence (Fig. 3C) of Ca\(^{2+}\). However, we cannot exclude the possibility that S-100 may act directly on MTs. Actually, preliminary data suggest that S-100 interacts with the elongation at pH 6.7 (20, 23).

In the presence of high Ca\(^{2+}\) (21), the available evidence indicates that, owing to the formation of S-100-tubulin complexes, a smaller percentage of tubulin molecules is available for MT formation and that the nearly complete disassembly due to sequestration of unassembled tubulin. Thus, the effect of S-100 on the nucleation than on the elongation of MTs (23) and in the absence (Fig. 3C) of Ca\(^{2+}\). However, we cannot exclude the possibility that S-100 may act directly on MTs. Actually, preliminary data suggest that S-100 interacts with the elongation at pH 6.7 (20, 23).

In the absence of S-100 we proposed that S-100 interferes with both the nucleation and the elongation of MTs (23) and in the absence (Fig. 3C) of Ca\(^{2+}\). However, we cannot exclude the possibility that S-100 may act directly on MTs. Actually, preliminary data suggest that S-100 interacts with the elongation at pH 6.7 (20, 23).

On the basis of kinetic data and on counts of MTs formed in the absence and presence of S-100 we proposed that S-100 interferes with both the nucleation and the elongation of MTs by sequestering unassembled tubulin (18, 23). The effect on elongation is most evident at pH 7.5, both in the presence (23) and in the absence (Fig. 3C) of Ca\(^{2+}\). However, we cannot exclude the possibility that S-100 may act directly on MTs. Actually, preliminary data suggest that S-100 interacts with the elongation at pH 6.7 (20, 23).

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