The effect of S-100a and S-100b proteins and Zn^{2+} on the assembly of brain microtubule proteins in vitro

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The homologous proteins S-100a and S-100b affect the microtubule system in a distinctly different way in the presence of low molar ratios of Zn^{2+}. Assembly of brain microtubule proteins can be almost completely inhibited and rapid disassembly can be induced by low molar amounts of S-100b in the presence of low molar ratios [2–4] of Zn^{2+}. Higher molar ratios per S-100b (>4) potentiate the general Zn^{2+} effect, promoting the formation of sheets of microtubules. However, the effect of S-100a is quite different, no inhibition of assembly can be observed and the presence of S-100a seems to protect the microtubule proteins against the effect of Zn^{2+} by chelating the Zn^{2+} and decreasing the free metal-ion concentration. S-100a or S-100b cannot bind to the microtubule polymer-form, either in the absence or in the presence of Zn^{2+}.

Microtubule assembly *Tubulin* *S-100 protein* *Calmodulin* *Zn^{2+} regulation*

1. INTRODUCTION

The cellular regulation of microtubule assembly and disassembly is unknown but divalent cations [1] may play an important role. Mg^{2+} is required for assembly of isolated microtubules and one Mg^{2+} is firmly bound to tubulin. On the other hand, Ca^{2+} in millimolar concentrations can induce disassembly and inhibit assembly. This effect is potentiated in the presence of Ca^{2+}-binding proteins like calmodulin, troponin and, as we recently reported, S-100 [2].

The brain S-100 protein is a mixture of two predominant components, the S-100a and S-100b proteins, which in bovine brain are present in almost equal amounts [3]. Both proteins are dimers of identical $\beta\beta$ (S-100b)- or non-identical $\alpha\beta$ (S-100a)-subunits. The α- and the β-subunit have a molecular mass of about 10500 and a high (58%) sequence homology [4]. Both subunits also share the typical amino acid sequence associated with a calcium-binding domain [5]. The biological activity of these proteins remains unknown. However, previous results [2] indicate that S-100 proteins regulate the calcium-induced microtubule disassembly in vitro.

It has recently been found that besides Ca^{2+} also Zn^{2+} can bind to the S-100a and to the S-100b protein and induce different conformational changes on both proteins [6]. It is well known that Zn^{2+} interferes with brain microtubules both in vitro [7] and in situ [8]. Tubulin has one high affinity site for Zn^{2+} [9]. Zn^{2+} in micromolar concentrations interferes with the tubulin–tubulin association as tubulin no longer assembles into microtubules but into sheets [7,10] in which the protofilaments are aligned antiparallel. Moreover, low Zn^{2+} concentrations, less than 0.1 mM, stimulate self-assembly of tubulin in the absence of MAPs [11]. Although small amounts of zinc have also been detected in isolated microtubule proteins these are probably associated with the microtubule-associated proteins, MAPs [12].

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As Zn^{2+} binds to S-100 proteins it was of interest to study the effect of Zn^{2+} on the microtubule system in the presence of the S-100 proteins. We here report that the S-100a and S-100b proteins induce quite different effects on the microtubule assembly in the presence of either micro- or millimolar Zn^{2+} concentrations.

2. MATERIALS AND METHODS

2.1. *Protein preparation*

Microtubule proteins and purified tubulin were prepared from bovine brain as in [13–15]. All assembly experiments were performed in assembly buffer: 100 mM piperazine-*N,N'*-bis(2-ethanesulphonic acid), 0.5 mM $MgSO_4$, 1 mM GTP, titrated to pH 6.8 with NaOH.

S-100a and S-100b protein were isolated from bovine brain by the technique in [3,16,17].

Calmodulin was isolated from bovine brain as in [3]. S-100 proteins and calmodulin were made calcium-free by trichloroacetic acid precipitation [18].

2.2. *Protein concentration*

The microtubule protein concentration was determined as in [13]. The concentration of S-100 was determined from E_{280} = 11500 for S-100a, from 3400 for S-100b [6] and 3300 $M^{-1} \cdot cm^{-1}$ for calmodulin [19].

2.3. *Microtubule assembly and disassembly*

Assembly was initiated by addition of concentrated microtubule proteins to S-100 protein and $ZnSO_4$ in the assembly buffer at 37°C and was monitored continuously by the change in absorbance at 350 nm [13–15]. Negatively stained samples and embedded pellets of assembled microtubules were studied by electron microscopy, as in [15].

2.4. *Immunological techniques*

The concentration of S-100a and S-100b in the supernatants were determined by rocket immunoelectrophoresis [20].

3. RESULTS

3.1. *Zn(II) protection by S-100a*

The rate and extent of assembly of microtubule proteins into microtubules were not affected by 30 μM metal-free S-100a (fig.1A,a'), which is a 2.5-times molar excess over tubulin dimer. Furthermore, neither the rate nor the extent of assembly were affected (fig.1A,b',d') in the presence of a 2.5-times molar excess of S-100a in the presence of 120 or 480 μM Zn^{2+} (either a 4- or 16-times molar ratio of Zn^{2+} over S-100a). Electron micrographs of the formed assembly product showed the presence of perfect microtubules with 4- or 16-times excess of Zn^{2+} over S-100a, while with a 32-fold excess, opened-up microtubules and an indication for sheets could be seen (micrographs not shown). Microtubule proteins assembled in the presence of the latter concentration of Zn^{2+}, but in the absence of S-100a showed an increased turbidity and the electron micrographs revealed the presence of sheets, confirming previous findings [7]. When 30 μM calmodulin was present with 240 μM Zn^{2+} no such protection as with S-100a could be observed. S-100a facilitates calcium-induced microtubule disassembly also in the presence of 4 Zn^{2+}/S-100a, as addition of 1 mM Ca^{2+} still could induce a rapid and nearly complete disassembly of microtubules [2].

The concentration of S-100a in the supernatant after pelleting of the microtubules formed in the presence of S-100a and a 4-times excess of Zn^{2+} was determined by rocket immunoelectrophoresis [19]. The supernatant was found to contain the same concentration of S-100a as before microtubule assembly, indicating that S-100a is not bound to the microtubules.

The self-assembly of phosphocellulose-purified tubulin induced by low concentrations of Zn^{2+} [11] was inhibited by equimolar amounts of S-100a.

3.2. *Zn(II) inhibition by S-100b*

Microtubule assembly is nearly completely inhibited in the presence of S-100b and low molar ratios of Zn^{2+} concentration as both the rate of assembly and the extent of assembly are decreased (fig.1B,b'). The inhibition seems to be Zn^{2+}-dependent (fig.2). Only a slight inhibition is found in the absence of Zn^{2+} with increasing amounts of S-100b as shown in fig.2. The effect may be due to a contamination of S-100b protein by Zn^{2+} which was isolated by a Zn^{2+}-dependent affinity chromatography [17]. This is also indicated by the complete assembly obtained in the

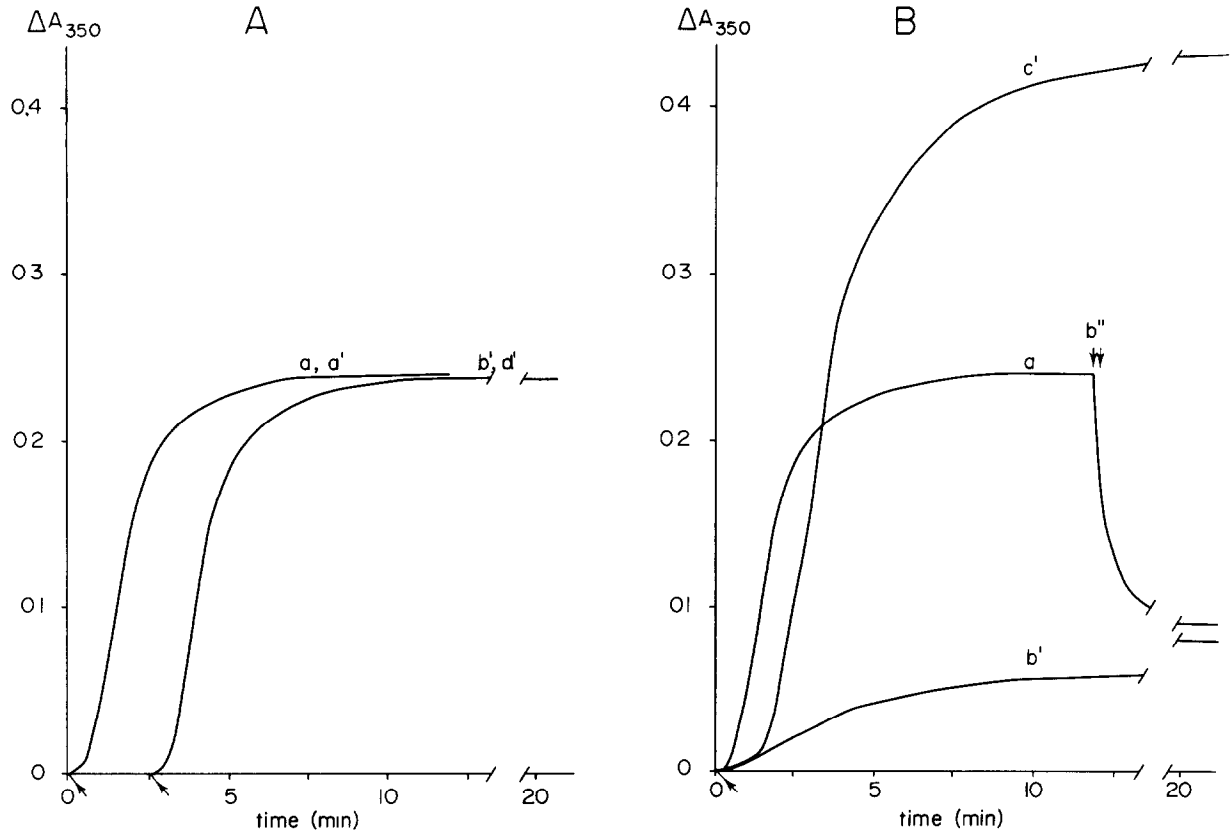

Fig.1. Microtubule assembly in the presence of Zn^{2+} and (A) S-100a and (B) S-100b protein. Microtubule proteins were assembled at 37°C in assembly buffer and monitored by the absorbance difference at 350 nm (ΔA_{350}), against time. The reference cell contained the same additions as the measuring cell but was kept at 10°C. At the arrow microtubule protein was added to the assembly buffer at 37°C. The microtubule protein concentration was 1.7 mg/ml of which the tubulin content was estimated to be 12 μM. (A) In the primed (') tracings 30 μM S-100a was present with: (a') 0 μM, (b') 120 μM and (d') 480 μM Zn^{2+}. Trace (a) was the control. (B) In the primed (') tracings 30 μM S-100b was present with: (a') 0 μM, (b') 120 μM and (c') 240 μM Zn^{2+}. At the double arrow (b'') 30 μM S-100b with 120 μM Zn^{2+} was added to the control (a).

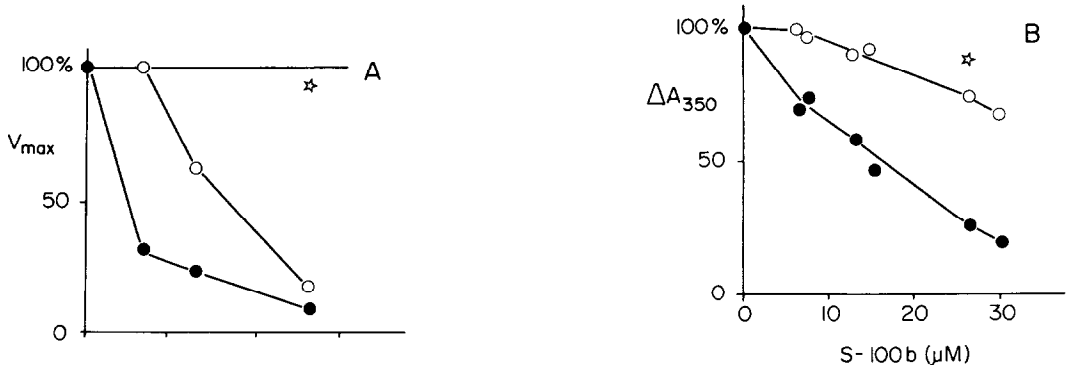

Fig.2. Assembly of microtubules in the presence of Zn^{2+} and S-100b. The conditions and microtubule protein concentration were as described in the legend to fig.1. (A) The maximal assembly rate (V_{max} = ΔA_{350}/min) is plotted against the added amount of S-100b; (B) the assembly level (ΔA_{350}) at steady state is plotted against the added amount of S-100b: (○) without Zn^{2+}; (●) with 4 Zn^{2+}/S-100b; (☆) with 1 mM EGTA.

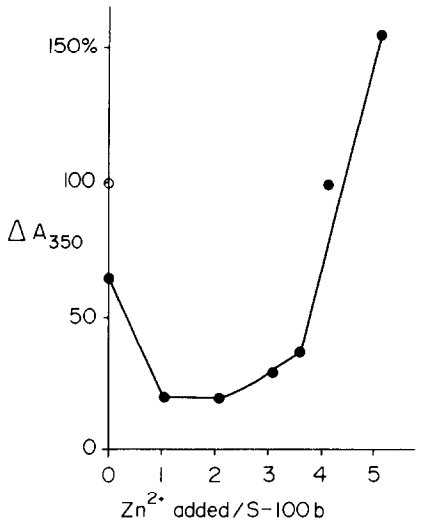

Fig.3. Assembly of microtubules in the presence of Zn^{2+} and S-100b. The assembly level (ΔA_{350}) at steady state is plotted against the added Zn^{2+}/S-100b. The concentration of S-100b was 0 μM (○) and 30 μM (●). The experimental conditions and microtubule protein concentration were as described in the legend to fig.1.

presence of S-100b when EGTA is added (fig.2 (*)). As can be seen in fig.1B and fig.2 and 3, the rate and the extent of assembly decrease in the presence of S-100b and Zn^{2+} at an optimum effect, see fig.3, with 2–4 Zn^{2+}/S-100b.

Furthermore, rapid disassembly of assembled microtubules is induced upon addition of S-100b with 2–4 Zn^{2+} (fig.1B,b′′). However, when this ratio is exceeded, microtubule assembly is enhanced (fig.1B,c′ and 3) and electron micrographs show the formation of sheets of microtubules (not shown).

The determination of the concentration of S-100b in the supernatant of the microtubules and microtubule sheets formed in the presence of 0, respectively 6 Zn^{2+} per S-100b showed that the S-100b concentration was about 95 ± 5% of the value of the reference without microtubular protein. These results indicate that S-100b does not bind to the tubulin polymer.

4. DISCUSSION

Although calmodulin and the S-100 proteins both have the amino acid sequences that could form the EF-hand [6] they exhibit different binding properties for divalent metal ions. Calmodulin binds 4 Ca^{2+} per molecule with dissociation constants in the range from 4–18 μM [18] and has low affinity for Zn^{2+} (K_d = 100 μM) [21]. In contrast, the affinity of S-100 for Zn^{2+} is higher than for Ca^{2+}, with 4 Zn^{2+} binding sites with dissociation constants in the range 0.01 μM and at least 4 more Zn^{2+}-binding sites with lower affinity (1 μM) [6]. Furthermore, there are significant differences in the Zn^{2+}-induced structural changes on the S-100a and S-100b proteins. S-100b becomes more hydrophobic in the presence of Zn^{2+}, while the hydrophobicity of S-100a is unaffected [6]. The present results show that an effect of Zn^{2+} and S-100 is found on the microtubule system with stoichiometric concentrations of Zn^{2+}, conditions under which calmodulin had no effect. S-100a and S-100b proteins have different effects on the microtubule disassembly. S-100a seems to protect the microtubules against the sheet-inducing effect of Zn^{2+} by decreasing the free Zn^{2+} concentration but not by binding to the microtubule proteins. Similar protective proteins have been observed in other tissues; e.g., metallothionein [22]. On the contrary, S-100b interacts with the microtubule proteins at low Zn^{2+} concentrations (2–4 Zn^{2+}/S-100b), induces disassembly and inhibits microtubule formation (fig.1–3). However, at higher Zn^{2+} concentrations, i.e., >4 Zn^{2+} per S-100b, the presence of S-100b enhances the general Zn^{2+} effect (fig.1B), inducing the formations of sheets. Thus, in the presence of the S-100b protein the inhibitory effect of Zn^{2+} on the microtubule assembly is within a narrow concentration range and may be correlated to specific Zn^{2+}-induced conformational changes of the S-100b protein. We have shown that the S-100 proteins are not incorporated in the microtubule polymer. However, this report confirms the interaction of the S-100 proteins with the microtubule system. It also demonstrates the different behaviour of the S-100a and S-100b proteins in the presence of Zn^{2+} which may suggest a different physiological role for both iso-proteins.

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