Effect of S-100 proteins and calmodulin on Ca^{2+}-induced disassembly of brain microtubule proteins in vitro

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1. INTRODUCTION

The brain-specific S-100 protein, discovered in 1965 [1] is a mixture of two very similar proteins, the S-100a and S-100b protein. These proteins are dimers of highly homologous subunits: S-100a ($\alpha\beta$) and S-100b ($\beta\beta$) [2,3]. Both proteins are small (M_r 20 000), very acidic and water soluble. It is assumed, that they are mainly located in the cytosol of glial cells [4] but they have also been found bound to membranes [5,6]. The biological activity of the S-100 proteins remains unknown. However, these proteins share two typical amino acid sequences in their primary structure, associated with the calcium-binding domain [7] which indicates that they also belong to the calcium-binding protein family, such as among others, calmodulin, troponin C and parvalbumin.

In our search for the function of S-100 proteins we have studied the effect of S-100 protein in a model system which required both the presence of Ca^{2+} and a calcium-sensitive effector. Brain microtubules have the ability to perform reversible cycles of assembly and disassembly [8]. This process has a central role in the ordered function of the cytoskeleton, as for example in cell movement and mitosis [9]. Microtubule protein is composed of a tubulin dimer and microtubule-associated proteins [8]. In vitro, microtubules disassemble into their subunits in the cold or in the presence of high concentrations of Ca^{2+} [8]. However, the concentration of free Ca^{2+} in the cell is rather low. Therefore, it has been proposed that a calcium-sensitizing factor or factors should regulate the microtubule disassembly in vivo [10,11]. Calmodulin has often been suggested [9,12,13] to have this role as it potentiates the disassembly effect of Ca^{2+} [12] and is found to be localized at the ends of the mitotic spindle [13]. We now report the effect of S-100 proteins on the Ca^{2+}-induced disassembly of microtubule proteins in comparison with the effect of calmodulin. We found that S-100 protein induced disassembly of microtubules with a higher efficiency than calmodulin at mM Ca^{2+} levels.

2. MATERIAL AND METHODS

2.1. *Protein preparation*

Microtubule proteins were prepared from bovine brain in the absence of glycerol by 2 cycles of assembly–disassembly [14,15]. All experiments were performed in the 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. The final pellet, which contains ~80% tubulin [14], was stored in liquid nitrogen. Prior to use, the pellet was re-suspended in assembly buffer and cycled once more [16].

S-100 protein was isolated from bovine brain by the technique in [16].

Calmodulin was isolated from ram testes by the procedure of [17] and was a generous gift of Professor J. Demaille. Calmodulin was freed of calcium by trichloroacetic acid precipitation [18].

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2.2. *Protein concentration*

The microtubule protein concentration was determined as in [14]. By ultraviolet spectroscopy the concentration of tubulin was calculated from $E_{278} = 1.2\ mg^{-1} \cdot cm^{-1}$ and M_r 110 000 of S-100 and calmodulin from $E_{280} = 8000$ [19] and 3300 [20] $M^{-1} \cdot cm^{-1}$, respectively.

2.3. *Microtubule assembly and disassembly*

Assembly of microtubule proteins in assembly buffer was started by increasing the temperature from 4–37°C and was monitored continuously by the change in absorbance at 350 nm [14,15]. Before assembly the microtubule proteins were incubated with different molar ratios of S-100 or calmodulin at 4°C. Disassembly was induced by addition of a concentrated solution of $CaCl_2$ to the measuring cell.

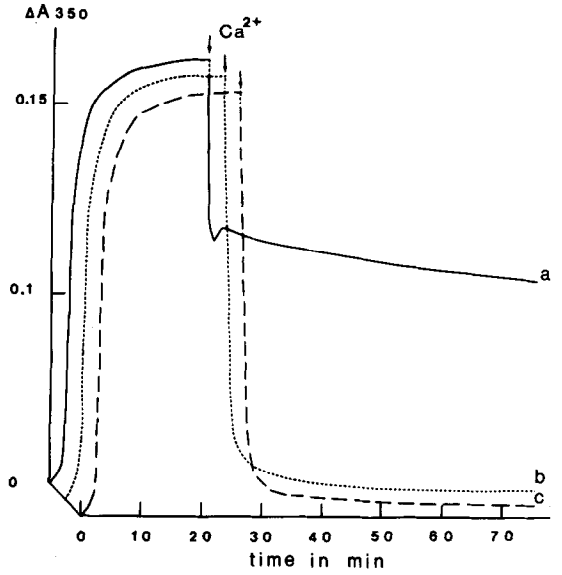

Fig.1. Ca^{2+}-dependent disassembly of microtubules in the presence of high concentrations of S-100 protein and calmodulin. Microtubule proteins were assembled at 37°C in buffer as monitored by the absorbance difference at 350 nm (A350), against time. The reference cell contained the same additions as the measuring cell but was kept at 10°C. When plateau turbidity was reached, after ~20 min, $CaCl_2$ to final conc. 1 mM was added (arrow). The protein samples were preincubated at 4°C for 15 min with (a) 0 = control; (b) 0.74 mg S-100/ml; (c) 1.6 mg calmodulin/ml. Microtubule protein was 1.6 mg/ml of which 80% is tubulin.

3. RESULTS

The rate and extent of assembly of microtubule proteins into microtubules were not affected by a molar excess of calmodulin or S-100 (7 and 3, respectively) to tubulin dimer, calculated on the basis of 80% tubulin and 20% microtubule-associated proteins (fig.1a–c). Assembled microtubules in the absence of calcium-binding proteins disassembled to ~40% upon addition of Ca^{2+} to 1 mM (fig.1a). However, when either S-100 or calmodulin were present addition of Ca^{2+} resulted in a rapid and complete disassembly of microtubules (fig.1b,c).

In contrast at a low molar ratio to tubulin dimer, 0.5, calmodulin has no longer effect on Ca^{2+}-induced disassembly (fig.2a,c). However, in the presence of 0.5 S-100/tubulin dimer, addition of 1 mM Ca^{2+} still induced a rapid and nearly complete disassembly (fig.2b). Furthermore, incubation of microtubule proteins with both 0.5 S-100/tubulin dimer and 1 mM Ca^{2+} completely inhibited assembly (not shown).

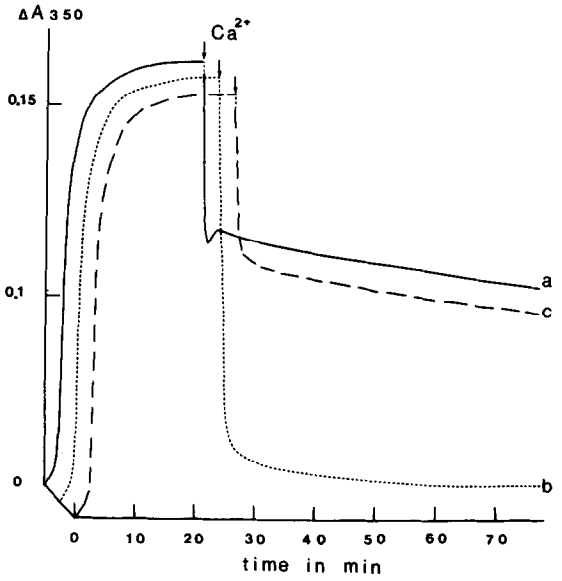

Fig.2. Ca^{2+}-dependent disassembly of microtubules in the presence of low concentrations of S-100 protein and calmodulin. The conditions are the same as in fig.1 but microtubule proteins were preincubated with (a) 0 = control, (b) 0.15 mg S-100/ml and (c) 0.11 mg calmodulin/ml.

4. DISCUSSION

Although calmodulin and the S-100 proteins both have the amino acid sequences that could form the EF-hand [7] they exhibit different Ca^{2+}-binding properties. Calmodulin binds 4 Ca^{2+}/molecule with dissociation constants in the range from 4–18 μM [18] whereas the S-100 proteins only have 2 Ca^{2+} binding domains with lower dissociation constant (50–1000 μM) [21]. The binding of Ca^{2+} to both proteins induces a conformational change, exposing hydrophobic groups to the solvent [19,21–24]. This property may explain the interaction with the microtubule. The high molar ratio of calmodulin to the tubulin dimer required to inhibit assembly in vitro makes it uncertain whether calmodulin regulates microtubule assembly in vivo even if the calmodulin concentration in the brain in considerable (1–20 μM) [9]. We report that the S-100 proteins which also are abundant in the brain can induce Ca^{2+}-dependent microtubule disassembly at a low molar ratio (0.5) to tubulin dimer. This might indicate a specific interaction of S-100 proteins with the microtubule system.

This work presents the first evidence for a measurable biological activity of the S-100 proteins in vitro. Some preliminary experiments with the purified S-100a and S-100b proteins have shown a slightly different effect on the microtubule disassembly (S-100a > S-100b). This may suggest a different physiological role for both isoproteins. Furthermore, the present findings may contribute to our knowledge of the functional relationship among the different Ca^{2+}-binding proteins and bring new perspectives for the understanding of microtubule assembly and disassembly in the brain.

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