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TUBULIN FROM *MIMOSA PUDICA* AND ITS INVOLVEMENT IN LEAF MOVEMENT

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Key Word Index—*Mimosa pudica*; *M. rubricaulis*; *Desmodium gyrans*; Leguminosae; tubulin; colchicine; colcemid.

Abstract—The sensitive plant *Mimosa pudica* is made insensitive by a brief treatment with colchicine. A high concentration of colchicine binding protein is present in the fresh actively moving leaves of *M. pudica*. This protein was partially characterized and compared with the animal brain tubulin. This colchicine binding activity is very low in the insensitive variety of *Mimosa*, namely *Mimosa rubricaulis*.

INTRODUCTION

Mimosa pudica L. is an interesting and unique species in the Plant Kingdom. Unlike most of the plants in general and other members of *Mimosa* it has rapid movement which is manifested in various different ways: (1) the main petiole droops down; (2) the pinnae come close together; and (3) the pinnules turn upward so that the members of each pair are brought in contact with each other. Increasing evidence has accumulated recently on the involvement of contractile proteins in various types of movement in non-muscle cells [1–5]. We have indirect evidence that contractile proteins are involved in the movement of the leaves of *M. pudica* by isolating an actin-like protein [6]. The presence of Mg^{2+}-dependent ATPase has been recorded from the actively moving leaves [7–9]. It is not yet clear whether this ATPase is associated with a contractile protein, like myosin. However, there are several instances of cell movement and cell shape changes in which a role of both microfilamets and microtubules in association with membranes has been suggested [3]. There are more subtle and recognized phenomena of movements of intrinsic membrane proteins that are certainly affected by drugs thought to be specific for the proteins of microfilaments and microtubules. It is known that the plant alkaloid colchicine binds specifically to tubulin, the subunit of microtubules, and can effectively interfere in the movement and shape of the cell [10]. Though microtubules have been well documented in plant cells, little is known of the properties of tubulin from higher plants due to the poor binding of colchicine with the tubulin-like protein from higher plants, compared to that of animal tubulin [11–15]. Recently, we observed that the leaves of *M. pudica* became completely insensitive to any stimulus on imbibition of colchicine (1 mM) for 4–6 hr at 25°. This effect can be reversed on removal of the plant from the colchicine solution, but on prolonged treatment the plant died. This observation led us to investigate whether the colchicine-binding protein, tubulin, is present in *M. pudica* in greater quantity compared to similar plants which do not show movement of leaves, and also to characterize the protein.

RESULTS AND DISCUSSION

The dialysed protein from fresh actively moving leaves of *M. pudica* was found to bind [^{3}H]colchicine. The colchicine binding property of the dialysed fraction was lost upon prior treatment with pronase. The binding increased linearly with protein concentration up to the capacity of GF/C filter discs as reported by Banerjee and Bhattacharyya [16]. The binding was also found to be a linear function of the concentration of colchicine up to 12.5 μM (data not given).

To test the specificity of [^{3}H]colchicine binding to the protein, it was incubated at 37° with 1×10^{-6} M [^{3}H]colchicine and applied to a Biogel P-10 column. A substantial amount of colchicine binding was obtained in the void volume of the column (Fig. 1A), and the elution profile was comparable to that obtained for goat brain tubulin binding to colchicine (Fig. 1B). The level of *M. pudica* protein bound radioactivity which appeared in the void volume could effectively be diminished if the protein loaded onto the column was incubated at 37° with a 500-fold excess of unlabelled colchicine together with [^{3}H]colchicine (Fig. 1A). However, this type of colchicine binding by extracts from other plants such as *Avena sativa* or *Phaseolus aureus* could not be demonstrated under similar experimental condition. This might be due to factors which inhibit this binding in those plants. Thus, specificity for colchicine binding to the *Mimosa* protein is indicated.

The time of equilibration for binding of colchicine (1 μM) to animal tubulin is reported to be 90–120 min

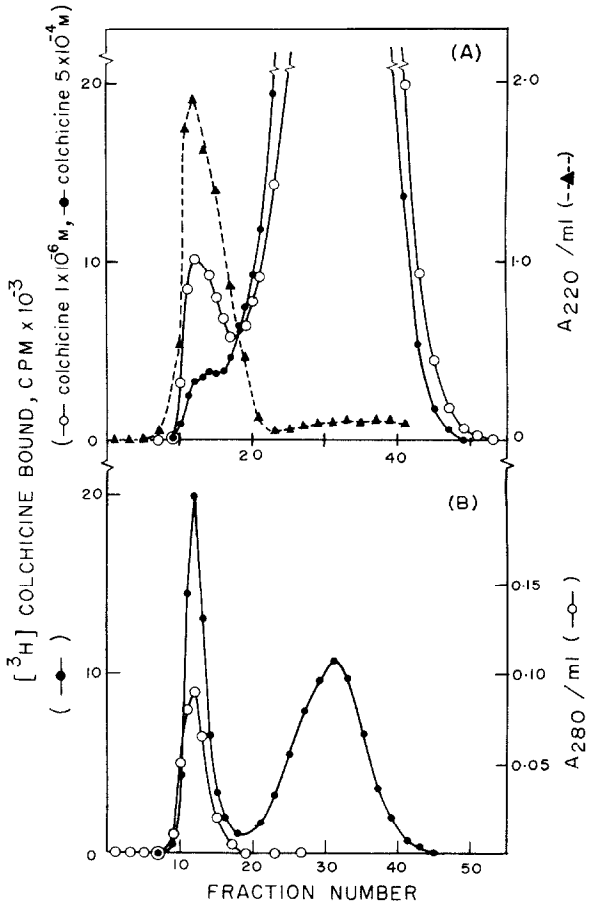

Fig. 1. Detection of colchicine binding activity in *M. pudica* protein and its comparison with brain tubulin. (A) 250 μl *M. pudica* protein (5.0 mg/ml) incubated at 37° for 60 min with either 1×10^{-6} M [^{3}H]colchicine alone in one set (○) or 1×10^{-6} M [^{3}H]colchicine plus 500-fold excess of unlabelled colchicine in the other set (●) was layered separately on a Biogel Pa10 column (25 × 1.2 cm) equilibrated with PMG buffer (pH 7). (B) 250 μl purified goat brain tubulin (1.48 mg/ml) treated as above, but with 1×10^{-6} M [^{3}H]colchicine (●) was layered onto the same column. After elution, the fractions (1 ml) were scanned for animal tubulin and the plant protein by monitoring the absorbance at 280 and 220 nm respectively. The plant protein has no absorption maximum at 280 nm [9]. 100 μl aliquots per fraction were soaked in GF/C paper, air-dried and scanned for radioactivity in toluene-based fluor.

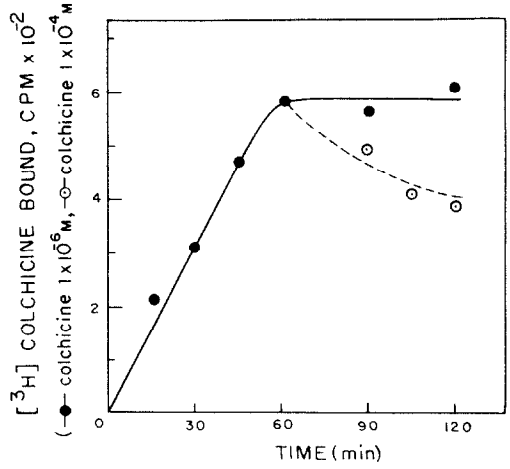

Fig. 2. Reversibility of colchicine binding. The plant protein (1.66 mg/ml) was incubated with [^{3}H]colchicine (1×10^{-6} M) at 37° for specified periods of time. After 60 min the appropriate amount of unlabelled colchicine was added to make the final concentration of ligand 1×10^{-4} M and incubated further at 37°. At the indicated periods aliquots were taken and assayed as described in the Experimental.

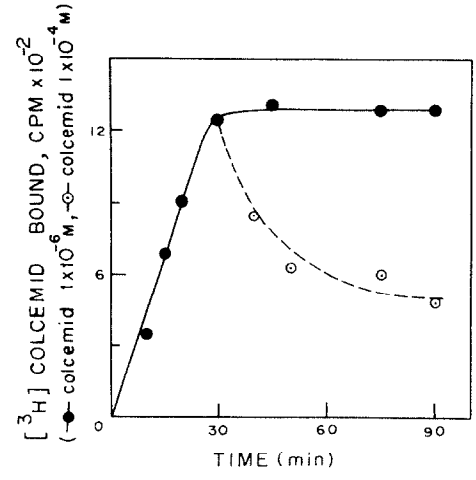

Fig. 3. Reversibility of colcemid binding. This experiment was like that shown in Fig. 2, using [^{3}H]colcemid (1×10^{-6} M). Unlabelled colcemid was added after 30 min incubation.

[17, 18]. Colcemid, the structural analog of colchicine, binds tubulin fairly rapidly [16] in *ca* 45 min. We have observed that the time of equilibration for binding of the plant protein to 1 μM colchicine and colcemid are 60 and 30 min respectively (Figs. 2 and 3). Bhattacharyya and Wolff [19] have shown that the binding of colchicine to rat brain tubulin is stimulated by certain anions such as sulfate and tartrate. It was interesting to find that binding of colchicine to the plant protein is also enhanced specifically by these anions (Table 1). It was further found that [^{3}H]colchicine binding to the protein increased linearly with increasing concentration of ammonium sulfate. The effect of other ions tested was not so pronounced. It was therefore apparent that the faster rate of binding of colcemid than that of colchicine, and the stimulation of colchicine binding by certain anions, as observed in the case of animal tubulins, were also characteristic properties of the *Mimosa* protein.

One of the most important properties of tubulin is its binding with the two drugs colchicine and colcemid, where the former binds irreversibly and the latter reversibly [16]. *M. pudica* protein previously

Table 1. Effect of anions on colchicine binding activity of the protein of *Mimosa pudica*

Sample	[^{3}H]Colchicine bound (cpm/mg protein)
Control	3190
$(NH_4)_2SO_4$	7920
Na_2SO_4	9760
Na-K-meso-tartrate	9520
NaCl	5470
NH_4Cl	4340

The plant protein (1.8 mg/ml) was incubated with [^{3}H]colchicine (1 μM) alone or with salt (0.6 M) at 37° for 60 min. The colchicine binding assay was performed as described in the Experimental.

incubated with [^{3}H]colchicine (1×10^{-6} M) at 37° for 60 min, when incubated further at 37° with a 100-fold excess of unlabelled colchicine, only 25% of the initial bound radioactivity was displaced within 1 hr (Fig. 2). On the contrary, in a parallel experiment using [^{3}H]colcemid (1×10^{-6} M) *ca* 60% of the initially bound radioactivity was displaced upon addition of a 100-fold excess of unlabelled colcemid (Fig. 3). These data clearly indicate that, unlike colchicine, the colcemid binding to *M. pudica* protein is highly reversible.

In order to determine whether the presence of colchicine binding protein was in any way related to the sensitivity of the plants, two other plants *Desmodium gyrans* and *Mimosa rubricaulis* were selected. *D. gyrans* which is also known as 'the telegraph plant,' has certain characteristic movements [20]. Unlike *M. pudica*, *M. rubricaulis* is insensitive to physical stimuli. The colchicine binding activity was measured using the dialysed ammonium sulfate pellet of leaf extract. It was found (Table 2) that *D. gyrans* leaves contain 64% and *M. rubricaulis* 17% of colchicine binding activity as compared to that in *M. pudica*.

A number of workers have already reported the presence of colchicine binding activity in plant extracts [11–15] which is comparable to that detected in the insensitive plant *M. rubricaulis*. On the basis of the greater amount of tubulin content (Table 2) and also the *in vivo* effect of colchicine on leaf movement, a possible role of tubulin in the movement of the leaves in *M. pudica* is suggested.

EXPERIMENTAL

Both colchicine and colcemid (ring C, [^{3}H]methoxy) having sp. act. 5.0 and 26.7 Ci/mmol, respectively were the products of New England Nuclear Corporation. GF/C filter paper was obtained from Whatman Ltd, England. All other chemicals used were reagent grade. PMG buffer: 10 mM KPi (pH 7), 10 mM $MgCl_2$ and 0.1 mM GTP, PM buffer: 10 mM KPi (pH 7) and 10 mM $MgCl_2$.

Extraction of protein. Fresh leaves of *M. pudica* were partially homogenized in a pestle and mortar and then completely in a Sorval Omnimixer with PMG buffer containing 0.25 M sucrose (w/v 1:2). The homogenate was strained through two layers of cheese cloth and then centrifuged at 10 000 *g* for 25 min. Ammonium sulfate was added to the supernatant to 85% satn and after 3–4 hr at 0°, the ppted protein was collected by centrifugation at 10000 *g* for 20 min. It was dissolved in PMG buffer and dialysed in 20 vol. of the same buffer for 90 min with two changes of buffer. All operations were performed at 0–4°. Protein concn was determined according to ref. [21] using bovine serum albumin as standard.

Binding assay. The GF/C filter disc assay for colchicine binding was carried out according to ref. [16]. For colcemid binding the method [16] was slightly modified to make it suitable for *M. pudica* protein. Whatman GF/C filter discs (two) were equilibrated with 1 ml cold (2–4°) PM buffer by suction. 1 ml PM buffer was applied to filters to which a 0.1 ml sample followed by 1 ml PM buffer were added and drained off slowly under mild suction. The filters were then washed ×3 with 3 ml cold PM buffer by mild suction, dried and counted in a toluene based fluor.

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Table 2. Detection of colchicine binding protein in sensitive and insensitive plants

Plants	Protein concentration (mg/ml)	[^{3}H]Colchicine bound (cpm/mg protein)
Mimosa pudica	1.84	5070
Desmodium gyrans	2.00	3280
Mimosa rubricaulis	2.00	865

Different amount of dialysed ammonium sulfate-pelleted proteins from different plants were incubated with [^{3}H]colchicine (1 μM) at 37° for 60 min. The colchicine binding assay was performed as described in the Experimental.

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