Lack of inhibition of carrot colchicine binding activity by podophyllotoxin

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Received 23 January 1984

The binding of [^{3}H]colchicine to carrot cell extract was not inhibited by an excess amount of podophyllotoxin. Under the same experimental condition, porcine brain tubulin almost completely lost its [^{3}H]colchicine binding activity. The components in the carrot cell extract did not affect the interaction of brain tubulin and podophyllotoxin.

Brain *Carrot* *Cell culture* *Colchicine* *Podophyllotoxin* *Tubulin*

1. INTRODUCTION

In [1–3], colchicine binding activity in carrot cell extract was investigated using the DEAE–Sephacel batch method. The properties of the colchicine binding component in the carrot extract were similar to those reported for brain tubulin [4,5], except that it was not inhibited by podophyllotoxin. We studied here the action of podophyllotoxin on the carrot components in more detail and compared it with that of brain tubulin.

2. MATERIALS AND METHODS

2.1. *Materials and cultivation*

Carrot cells of strain GD2 [6] were grown in a modified medium of [7]. Colchicine was obtained from Merck and podophyllotoxin from Aldrich. [^{3}H]Colchicine, ^3H_2O and [^{14}C]colchicine were purchased from New England Nuclear. Lumicolchicine was prepared as in [1].

2.2. *Preparation of brain tubulin and carrot cell extract*

Porcine brain tubulin was prepared by two cycles of assembly and disassembly, essentially as in [8]. The tubulin was dissolved in 1 M tartrate in PMg buffer (10 mM K–phosphate, 10 mM $MgSO_4$, pH 6.9) and dialyzed against the same buffer overnight before use. Carrot cell extract was prepared from exponentially growing culture as in [3]. The [^{3}H]colchicine binding activity was assayed by the DEAE–Sephacel batch method [3].

3. RESULTS AND DISCUSSION

3.1. *Lack of inhibition of carrot colchicine binding activity by podophyllotoxin*

The preparation obtained from the carrot cells contained colchicine binding component which was not contaminated with lumicolchicine. Podophyllotoxin, which is known to be an inhibitor of colchicine binding to brain tubulin [4,5], showed little or no effect on binding of colchicine to the carrot cell component (table 1). In the same assay system, [^{3}H]colchicine binding to brain tubulin was almost completely inhibited by preincubation with podophyllotoxin (table 2; exp.1,3). One may believe that the binding site of the carrot component becomes occupied by colchicine during the incubation period if its binding reaction to podophyllotoxin is highly reversible compared to that to colchicine. However, this is unlikely because the time course of the colchicine binding

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Table 1

[^{3}H]Colchicine binding to carrot cell extract after preincubation with non-radioactive colchicine, lumicolchicine and podophyllotoxin

Drug	[^{3}H]Colchicine bound		
	dpm/mg protein	pmol/mg protein	%
Control	2833	6.38	100
Colchicine	444	1.00	15.7
Lumicolchicine	2745	6.18	96.7
Podophyllotoxin	2817	6.35	99.2

After aliquots of carrot cell extract (6.75 mg protein/0.45 ml) were preincubated at 37°C for 30 min with the non-radioactive drugs indicated above, [^{3}H]colchicine was added to each mixture and incubation continued for 1.5 h. Concentrations of non-radioactive drugs and [^{3}H]colchicine in the final mixture were 100 and 5 nmol per ml, respectively

reaction after preincubation with podophyllotoxin was similar to that without podophyllotoxin.

3.2. *Effect of podophyllotoxin on brain tubulin in the presence of carrot extract*

It is possible that some contaminants in the carrot cell extract might decrease the inhibitory effect of podophyllotoxin or alter the podophyllotoxin sensitivity of brain tubulin. This was tested by preincubation of carrot cell extract with either podophyllotoxin or brain tubulin prior to [^{3}H]colchicine binding assay (table 2; exp.4–7). In every case, [^{3}H]colchicine binding to brain tubulin was inhibited by podophyllotoxin. Taking into account the contribution of colchicine binding activity of carrot extract which is unaffected by podophyllotoxin, the results in table 2 show that the preincubation with carrot extract has little effect on the property of brain tubulin or podophyllotoxin. These results suggest that the insensitivity of the carrot colchicine binding component to podophyllotoxin is an intrinsic property rather than a consequence of the reaction with contaminants. To confirm that the observed colchicine binding activity does not represent unspecific substances other than tubulin, the following experiments were performed. Table 3 shows that the colchicine binding component in carrot cell is heat-

Table 2

Inhibitory effect of podophyllotoxin on [^{3}H]colchicine binding to brain tubulin after preincubation of brain tubulin or podophyllotoxin with carrot extract

Exp.	Preincubation			Incubation		[^{3}H]Colchicine bound	
	C.E.	B.T.	Podo.	B.T.	Podo.	dpm	%
1	−	+	−	−	−	181657	100
2	−	+	−	−	+	4175	2.3
3	−	+	+	−	−	2383	1.3
4	+	−	−	+	−	192484	106
5	+	+	−	−	+	17608	9.7
6	+	−	+	+	−	18303	10.1
7	+	+	+	−	−	16950	9.3

Preincubation was performed at 37°C for 30 min, thereafter incubation was carried out at 37°C for 1.5 h with [^{3}H]colchicine (5 nmol, 1 μCi/ml in the final mixture); conditions: 0.225 ml of the 100000 × g supernatant of the carrot cell extract (C.E., 3.6 mg protein); 0.25 ml of porcine brain tubulin (B.T., 0.125 mg protein); podophyllotoxin (Podo.), 100 nmol/ml in the final mixture; %, relative values to the amount of bound [^{3}H]colchicine in exp.1

labile and susceptible to proteolytic enzymes. When [^{3}H]colchicine-labelled brain tubulin and [^{14}C]colchicine-labelled carrot component were mixed and applied on a DEAE–Sephacel column and subsequently on a Sephadex G-200 column

Table 3

[^{3}H]Colchicine binding to carrot cell extract after treatment with heat or protease

Preincubation		[^{3}H]Colchicine bound		
Treatment	Temperature (°C)	dpm/mg protein	pmol/mg protein	%
Control	37	2950	6.64	100
Heat	57	616	1.39	20.9
Pronase	37	49	0.11	1.7
Trypsin	37	147	0.33	5.0

After carrot cell extract (6.3 mg protein/0.45 ml) was preincubated at 57°C (heat) or at 37°C with protease (0.5 mg/ml final concentration) for 30 min as indicated above, [^{3}H]colchicine was added to each mixture and incubation continued for 1.5 h at 37°C

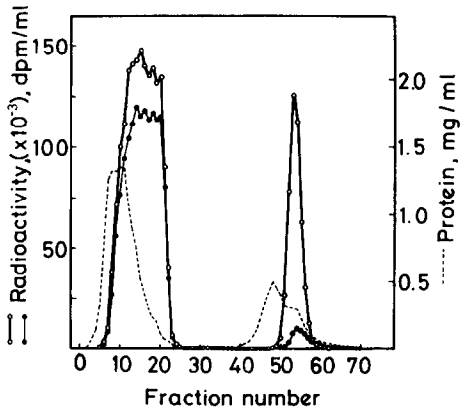

Fig.1. Co-chromatography of [^{3}H]colchicine-bound brain tubulin and [^{14}C]colchicine-bound component in carrot cell extract on a DEAE–Sephacel column. [^{3}H]Colchicine-bound brain tubulin [(○—○) 0.44 mg protein/0.5 ml] and [^{14}C]colchicine-bound component in carrot cell extract [(●—●) 7.75 mg protein/0.5 ml] were prepared separately and applied on a DEAE–Sephacel column immediately after mixing. The column was eluted first with 0.1 M NaCl in PMg buffer (40 ml) and then with 0.1–2 M NaCl in PMg buffer (50 ml:50 ml).

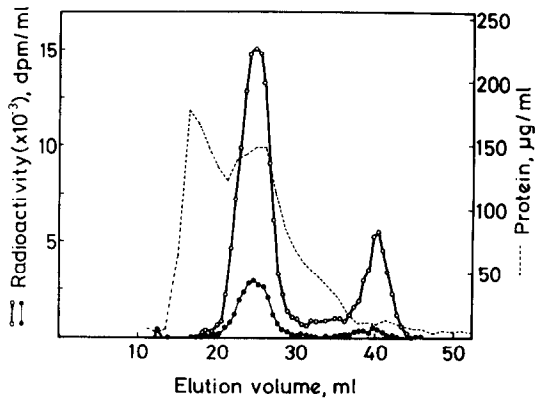

Fig.2. Co-chromatography of [^{3}H]colchicine-bound brain tubulin and [^{14}C]colchicine-bound component in carrot cell extract on Sephadex G-200 gel column. Fractions 50–57 in fig.1 were collected, concentrated and applied on a Sephadex G-200 column (11.7 × 350 mm).

(fig.1,2), the elution pattern of brain tubulin and carrot component was very similar.

These results indicate that the colchicine binding component in carrot cell extract is a similar protein to brain tubulin but not identical with respect to the reactivity with podophyllotoxin.

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