COLCEMID AND COLCHICINE BINDING TO TUBULIN

Similarity and dissimilarity

Ambica C. BANERJEE and B. BHATTACHARYYA
Department of Biochemistry, Bose Institute, Calcutta 700 009, India

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1. Introduction

A unique property of tubulin is to bind colchicine and colcemid, two structurally similar drugs, both of which disaggregate the polymerised form of this protein [1–5]. In spite of structural similarity, certain differences in mode of action for these ligands exist. Studies on mitotic arrest of different cells in culture with colchicine and colcemid suggest a more rapid release of bound colcemid than of colchicine [6]. Moreover, the response of yeast tubulin towards colcemid binding and not to colchicine [7], led us to investigate the mechanism of binding of these two structurally similar drugs to tubulin. Here we describe experiments to show that binding kinetics of colchicine and colcemid to tubulin are very different.

2. Materials and methods

Both colchicine and colcemid (ring C, [^{3}H]methoxy) having spec. act. 5 Ci/mmol and 9.3 Ci/mmol, respectively, were products of New England Nuclear Corp. GTP (Grade IIS) and colchicine were products of Sigma. Colcemid was obtained from K and K. GF/C and DE 81 filter papers were products of Whatman. All other chemicals used were reagent grade.

Tubulin was purified from goat brain according to [8] and stored at −10°C in PMG (10 mM phosphate (pH 7.0), 10 mM $MgCl_2$ and 0.1 mM GTP) buffer containing 1 M sucrose. Protein was determined bythe Lowry method [9] using bovine serum albumin as standard.

2.1. *Binding assay*

The DE 81 filter paper disc assay for colchicine [10] had to be modified to make it suitable for colcemid binding. Whatman GF/C filter paper discs (one or more depending on the protein content) were washed with 1 ml cold (2–4°C) PM buffer (10 mM phosphate (pH 7.0) and 10 mM $MgCl_2$) by suction, taking care not to dry the papers. The sample (0.1 ml) was applied and absorbed to the filters directly, over 1–2 min. The filters were then rinsed 3 times with 3 ml cold PM buffer by mild suction, dried and counted in toluene-based fluor.

3. Results

Preliminary experiments as shown in table 1 suggest that although both colchicine and colcemid possess identical trimethoxy phenyl and tropolone moieties, the DE 81 filter disc assay for colchicine could not be used to study colcemid–tubulin interaction. We have therefore developed a new assay method to study colcemid–tubulin interaction using glass fibre filter paper which could be successfully used in the case of colchicine tubulin interaction too. Formation of colcemid–tubulin complex, detected by the new GF/C filter paper disc method described, is a linear function of protein concentration, as shown in fig.1.

The rate of colchicine binding to tubulin is very

Table 1
Colchicine and colcemid binding to tubulin as compared by DE 81 and GF/C filter disc methods

Conditions	% Total input radioactivity retained on filter papers			
	DE 81		GF/C	
	[^{3}H]Colchicine	[^{3}H]Colcemid	[^{3}H]Colchicine	[^{3}H]Colcemid
No Tubulin	0.8	4.0	0.75	1.0
100 μg Tubulin	11.0	6.0	9.0	6.0

Tubes were incubated with labelled drugs (1 μM) at 37°C for 1 h with and without tubulin

slow and has high temperature coefficient. The binding requires long incubation at 37°C to reach the equilibrium [5]. Figure 2 shows that the binding of colchicine (1 μM) to tubulin required about 2 h to equilibrate at 37°C (fig.2A), whereas, the binding of colcemid (1 μM) occurred at a much faster rate requiring only about 45 min to reach the equilibrium (fig.2B). It may also be noted from the figure that at time zero, [^{3}H]colchicine did not bind tubulin at all, but [^{3}H]colcemid binding occurred to ~40% of its total value.

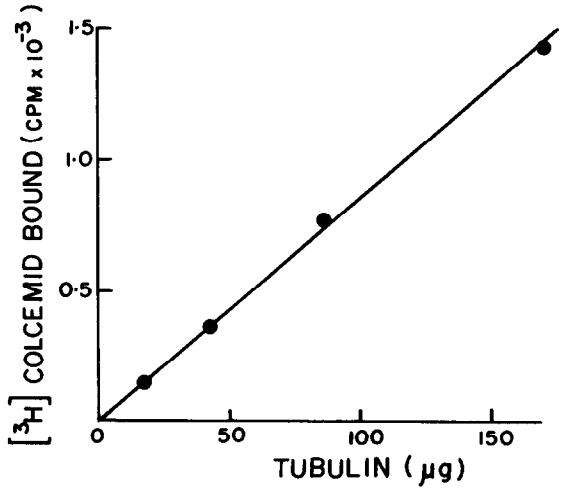

Fig.1. Linearity of the GF/C filter disc assay for the colcemid–tubulin binding reaction. [^{3}H]Colcemid (1 × 10^{-6} M) was incubated with increasing concentration of tubulin in PMG buffer (pH 7.0) for 45 min at 37°C.

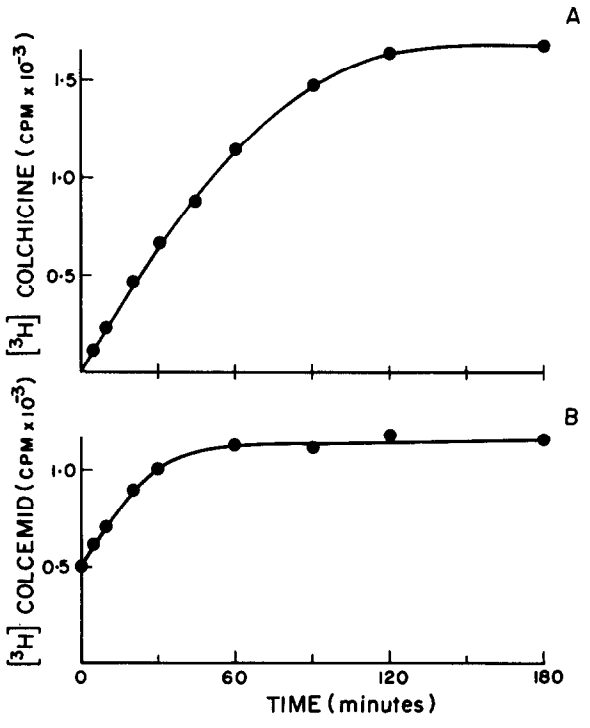

Fig.2. Time dependence for the binding of [^{3}H]colchicine and [^{3}H]colcemid to tubulin. Tubulin (0.7 mg/ml) was incubated with (A) [^{3}H]colchicine (1 μM) and (B) [^{3}H]colcemid (1 μM) at 37°C. At the times indicated aliquots from each tube were removed and assayed for the bound radioactivity by the GF/C filter disc method as described.

Table 2
Effect of excess unlabelled colcemid and colchicine on their respective complexes with tubulin

Incubation period after addition of unlabelled drug (min)	% Initial binding remaining	
	Colcemid	Colchicine
0	100	100
10	66.3	96.6
20	55.1	90.4
30	42.1	92.6
60	34.2	87.9
120	27.3	82.8

Tubulin (0.7 mg/ml) was incubated with [^{3}H]colcemid (1 μM) and [^{3}H]colchicine (1 μM) separately for 1 h at 37°C. Appropriate amounts of unlabelled drugs were added to the respective tubes to make 1 × 10^{-4} M ligand final conc. and incubated further at 37°C. At indicated periods of time aliquots were taken and assayed using GF/C filter paper method as described

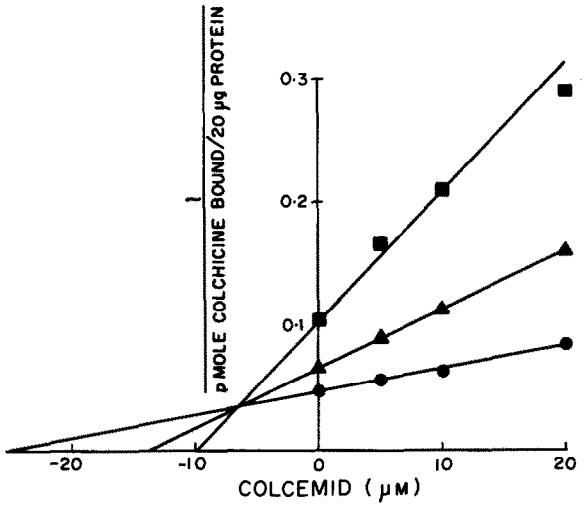

Fig.3. Modified Dixon plot demonstrating the effect of colcemid on the colchicine binding to tubulin. The concentration of colcemid present is plotted against the reciprocal of the bound [^{3}H]colchicine at several concentrations of [^{3}H]colchicine: (■) 1 μM; (▲) 2 μM; (●) 4 μM. The reaction mixtures containing tubulin (0.2 mg/ml) and the drugs as indicated were incubated at 37°C for 90 min and binding was assayed by the GF/C filter disc method as described.

Another important difference in the binding of these two ligands to tubulin is that, while one is almost irreversible the other one is freely reversible. Table 2 shows that when an excess of unlabelled colcemid (1 × 10^{-4} M) is added to tubulin that has been preincubated with [^{3}H]colcemid (1 μM) and is incubated further, ~66% of the total initial bound radioactivity is displaced within 1 h. In contrast, the initial radioactivity bound to tubulin in a parallel experiment with [^{3}H]colchicine (1 μM) remains largely unaffected upon the addition of 1 × 10^{-4} M unlabelled colchicine. This suggests that unlike colchicine, the binding of colcemid to tubulin is highly reversible.

So far we have furnished evidence to show that the binding of colcemid to tubulin differs from that of colchicine with respect to rapid equilibration, time zero binding and finally, reversibility. Next, we examined the effect of colcemid on the colchicine binding site of tubulin as analysed by modified Dixon plot. Figure 3 shows that the [^{3}H]colchicine binding is inhibited competitively by colcemid yielding an app. K_i 6.5 × 10^{-6} M. Thus, although the mechanism of colcemid binding to tubulin differs from that of colchicine, both the drugs appear to bind tubulin at the same binding site.

4. Conclusion

Here we have confirmed the conclusion that colcemid is a competitive inhibitor for colchicine binding to tubulin. However, the two binding processes were found to be very different in the following properties:

(i) Colchicine binds tubulin slowly and requires 2 h to attain equilibrium at 37°C, whereas, colcemid binds more rapidly and equilibrium is attained within 45 min;
(ii) In contrast to colchicine–tubulin interaction, which is almost irreversible, the binding of colcemid to tubulin is freely reversible;
(iii) Moreover, though colchicine does not bind tubulin at 0°C, there is substantial binding of colcemid to tubulin at this temperature.

These differences made it difficult to envisage a single mechanism for the apparent competition between these two ligands for the same binding site. Whether these imply that the mechanism of binding reaction of these two ligands to the same site of

tubulin will be determined by the substituents in the B-ring remains to be settled.

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