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Colchicine binding activity of rat brain polysomes

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Abstract. In this communication, we report the presence of a unique colchicine-binding activity in the polysomes of rat brain. This drug-binding property, is somewhat similar to that of tubulin isolated from many sources; however, it differs in several biochemical characteristics such as (i) thermal stability of colchicine-binding site, (ii) protection of binding site by vinblastine and (iii) time required for binding equilibration. Such binding of colchicine to the polysomes is most probably due to the presence of a nascent peptide chain of tubulin in the polysome.

Keywords. Colchicine-binding activity; tubulin polysomes; nascent peptide chain.

Introduction

Colchicine binds to tubulin, the subunit of microtubule with a stoichiometry approaching one mol of alkaloid per mol of tubulin dimer. This unique binding specificity of colchicine tubulin interaction which is comparable to enzyme-substrate reaction has been routinely employed to characterise tubulin (Olmsted and Borisy, 1973; Wilson and Bryan, 1974; Bhattacharyya and Wolff, 1974, 1975, 1976, 1976a). The ubiquitous and multifunctional nature of tubulin commends it for studies on the regulation of its biosynthesis and function. Vertebrate brain constitutes a rich source of tubulin and is very likely to contain also an appreciable amount of tubulin-specific messenger RNA. The approach for the isolation of tubulin mRNA is still very limited except for a few reports (Cleaveland *et al.,* 1978; Gozes *et al.,* 1980; Portier *et al.,* 1980). The purification of a specific mRNA requires selection of specific polysomes with which the mRNA of the desired proteins is associated. In the present study, colchicine is used to detect the tubulin synthesizing polysomes isolated from rat brain. This colchicine binding property of the brain polysomes is characterized in order to compare with that of tubulin itself, the protein isolated from many sources.

Materials and methods

Radioactive colchicine (ring C[^{3}H]methoxy) having specific activity 5 Ci/mmol was the product of New England Nuclear Corpn., Boston, Massachhusetts, USA.

Abbreviations used: TNMG, Tris-HCl buffer pH 7.2,25 mM NaCl, 5 mM $MgCl_2$ and 0.1 mM GTP: UV; ultra violet; DEAE, diethyl aminoethyl.

GTP (Grade IIS) and colchicine were products of Sigma Chemical Co., St. Louis, Missouri, USA. Colcemid was a kind gift from Dr. Jan Wolff of National Institutes of Health, Bethesda, Maryland, USA. DE-81 and GF/C filter papers were obtained from Whatman Inc., UK. All other chemicals used were reagent grade.

Polysomes were isolated from rat brain (1 month old) according to the method of Pemberton *et al.* (1972) with little modifications. Excised brain was homogenized (4 ml/g) with 10 mM Tris-HCl buffer (pH 7.4), 10 mM KCl and 1 mM $MgCl_2$. The crude homogenate was mixed with 1/3 volume of 2.4 M sucrose, 170 mM Tris-HCl buffer (pH 7.4), 70 mM KCl and 1 mM $MgCl_2$. The nuclei were removed by centrifugation at 5000 *g* or 10 min. The postnuclear supernatant was centrifuged at 30,000 *g* for 10 min to remove mitochondria. Free polysomes were recovered by centrifugation of the postmitochondrial supernatant at 100,000 *g* for 3.5 h through a sucrose cushion contaning 1.6 M sucorse, 50 mM Tris-HCl buffer (pH 7.4), 250 mM KCl and 2 mM $MgCl_2$.

Binding of [^{3}H] colchicine to rat brain polysomes

The binding of labeled colchicine to polysomes was quantitated by the method of Bhattacharya and Wolff (1975). DEAE-filter paper discs were initially washed with 1 ml of cold (2–4°C) PM buffer (10 mM potassium phosphate, pH 7.0, and 10 mM $MgCl_2$), taking care not to dry the papers. Then, 1 ml of the buffer containing 10 µm colchicine was poured on the filter paper to which 100 µ1 of the sample was added and allowed to drain off under mild suction. The filters were then rinsed 3 times with 3 ml of the cold buffer by mild suction, dried, and counted in 5 ml of toluene-based fluor. Identical blanks, without polysome, were prepared and necessary corrections were made.

Results

Polysome profile

The polysomes were suspended in TNMG buffer (25 mM Tris-HCl buffer, pH 7.4, 25 mM NaCl, 5 mM $MgCl_2$ and 0.1 mM GTP), layered over a linear sucrose (15–30%) gradient and centrifuged at 25,000 *g* for 90 min. Fractions were collected using an ISCO Model 183 fractionator and the absorbance at 254 nm was recorded with an UA-5 ultra violet (UV) monitor. The profile of the polysomes shows the integrity of the isolated polysomes (figure 1). Preliminary binding experiments with this purified preparation suggest that like tubulin from several sources polysomes isolated from brain, bind [^{3}H] colchicine (table 1) and that the binding activity is a linear function of the polysome concentration (figure 2a) with a temperature optimum at 37° C (figure 2b). The specificity of this binding is also similar to that of tubulin from brain and other sources (Olmsted and Borisy, 1973; Wilson and Bryan, 1974; Wolff and Williams, 1973). Thus, the binding of colchicine to brain polysomes can be completely blocked, if the polysomes are preincubated with other drugs, that are known to bind specifically at the colchicine-binding site of tubulin (table 1). However, unlike colchicine-tubulin interaction which is a slow process and requires 2 h to attain equilibrium at 37°C, the rat brain polysomes bind colchicine more rapidly and equilibrium is attained within 45 min (data not shown). Although, the soluble tubulin constitutes the bulk of the colchicine-binding activity of many tissues (Borisy and Taylor, 1967a, b), it has

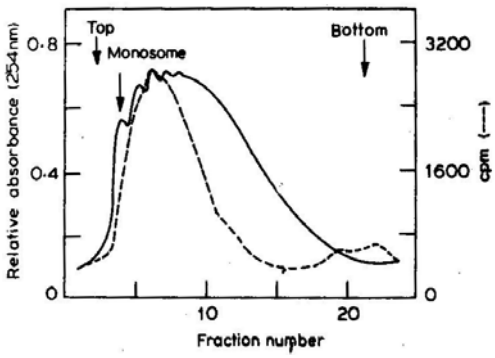

Figure 1. Sedimentation profile of the polysomes. Approximately 70 A_{260} units in 0.5 ml were applied on 15–30% sucrose gradients. Centrifugation was at 25,000 rpm for 90 min at 0° C in a Spinco ultracentrifuge.

Table 1. Effect of colchicine analogues on [^{3}H]colchicine binding to rat brain polysomes.

Experiments	[^{3}H] colchicine binding activity (cpm/2.08 A_{260} units)
Control	1329
Preincubated with colchicine (0.1 mM)	93
Preincubated with colcemid (0.1 mM)	128
Preincubated with podophyllotoxin (8.12 mM)	83

Polysomes (5.2 A_{260} units) were preincubated with drugs for 10 min at 37° C and the colchicine binding activity were measured by further incubating the samples with [^{3}H]colchicine (1, μM) at 37° C for 30 min.

been shown that the colchicine-binding protein may also be associated with the particulate cell fractions (Feit and Barondes, 1970; Dahl *et al.,* 1970; Lagnado *et al.,* 1971; Stadler and. Franke, 1972). Thus, the question arises whether this colchicine-binding activity, residing in the brain polysomes, is due to a specific association with nascent tubulin, or due to some nonspecific adsorption of cytoplasmic soluble tubulin or plasma membrane bound tubulin during cell homogenization. To test these possibilities, cytoplasmic tubulin-[^{3}H] colchicine complex was added before homogenizing the brain and polysomes were prepared in the usual procedure. There was no detectable radioactivity in the polysomes after separation through a discontinuous sucrose gradient (data not shown). This result eliminates the possibility of contamination of the polysomes with the cytoplasmic tubulin. Furthermore, since the concentration of sucrose cushion (1.6 M) used in the isolation of polysomes is more than 45%, it seems unlikely that the colchicine-binding activity of the polysomes was due to the contamination by the plasma membrane or an artifact of separation (Bhattacharyya and Wolf, 1975).

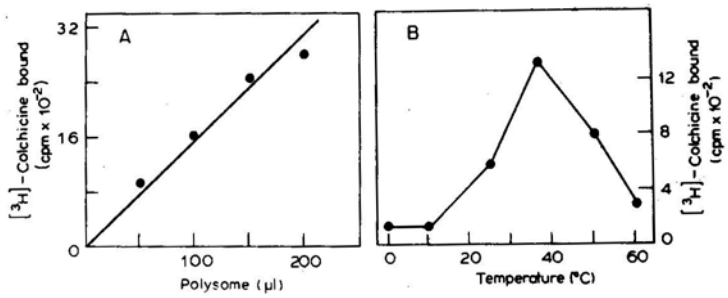

Figure 2. Binding of [^{3}H]colchicine to rat brain polysomes. **A.** Linearity of the [^{3}H] colchicine-polysome binding reaction. [^{3}H] Colchicine (1 µm) was incubated with increasing concentrations of polysomes in a final volume of 250 µl, at 37° C for 30 min and the binding was monitored as described in methods. **B.** Temperature dependence of [^{3}H] colchicine-polysome binding reaction. Aliquots of polysome (5.2 A_{260} units) in a final volume of 250 µl were incubated with [^{3}H] colchicine (1 µm) at different temperatures for 30 min and colchicine binding was measured.

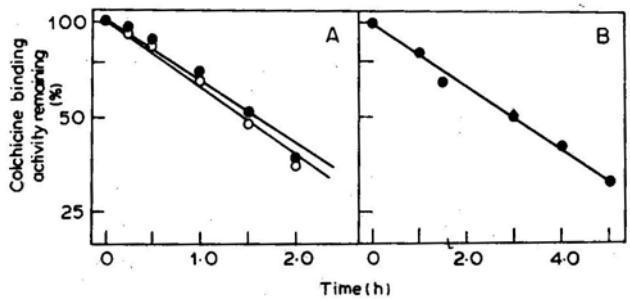

Figure 3. Time decay of colchicine-binding activity of rat brain polysomes. **A.** Aliquots of' polysomes (5.2 A_{260} units) in a final volume of 250 µl were incubated in the absence (O) or in the presence (●) of 10 µM vinblastine sulphate at 37°C for various periods. Colchicine binding activity of the preincubated samples were determined by further incubating with [^{3}H] colchicine 1 µM at 37° C for 30 min. Data were expressed as the per cent binding activity remaining compared to the sample without preincubation as 100%. **B.** Aliquots of polysomes (5.2 A_{260} units) were incubated at 0° C for various periods. Colchicine binding activity of the samples were determined by further incubating the samples with [^{3}H]colchicine 1 µM at 37° C for 30 min.

An interesting property of the colchicine-binding site of tubulin is its lability at 37° C. Figure 3a shows the decay characteristics of colchicine-binding activity of polysomes at 37° C. In comparison to soluble and membrane-bound tubulins (Bhattacharyya and Wolff, 1975), the colchicine-binding activity of the brain polysomes is more liable at 37° C. The time required for the loss of 50% binding activity over the control has been found to be only 90 min whereas the values for the soluble and the membrane bound tubulins are 4 h and 7 h respectively (Bhattacharyya and Wolff, 1975). Moreover, vinblastine which is known to protect the colchicine-binding site of tubulin (Bhattacharyya and Wolff, 1975; Wilson, 1970), fails to stabilise the colchicine-binding activity of the polysomes (curve 2). It has already been known that tubulin isolated from different sources is quite stable at 0° C with respect to the colchicine binding. But surprisingly this polysomal colchicine-binding activity is so labile that it decays even at 0°C (figure 3b), showing a half-

life of 3 h. It seems, therefore that the colchicine-binding activity of polysomes is not due to any contamination of soluble or membrane bound tubulin. Such binding of colchicine to the polysomes is most probably due to the presence of nascent peptide chain of tubulin in the polysome. However, our efforts to release the nascent chain by puromycin treatment to show colchicine binding activity failed. Since the colchicine binding activity of polysome is highly labile compared to cytoplasmic tubulin (figure 3b), it is possible that puromycin released subunit quickly lost its conformation (needed for the colchicine binding activity) when present in a monomeric form.

Discussion

In this communication, we report the presence of a unique colchicine-binding activity in the polysomes of rat brain. This drug-binding property, is somewhat similar to that of tubulin isolated from many sources; however, it differs in several biochemical characteristics such as (i) thermal stability of colchicine-binding site, (ii) protection of binding site by vinblastine and (iii) time required for binding equilibration. Such binding of colchicine to the polysomes is most probably due to presence of nascent peptide chain of tubulin in the polysome. However, it is not possible to suggest as to whether α or β subunit binds colchicine *per se.* After addition of colchicine before fractionation of polysomes it is noted that the profile of colchicine binding remained the same (data not given). Had it been due to both the subunits, a shifting of colchicine binding profile of polysomes towards bottom should have been noted. Nevertheless, the most important finding which emerges from this study is that since the polysome-bound nascent peptide binds colchicine and α and β subunits of tubulin are coded by two different genes as reported by Valenzuela *et al.* (1981), the colchicine-binding site of tubulin is expected to be located in one of the subunits. The present results support this fact. Recently similar conclusion was also drawn by Rousset and Wolff (1980) on the basis of their studies on lactoperoxidase-tubulin interaction. Finally, colchicine-binding of polysome might be used as a probe for isolation of specific tubulin mRNA.

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