Isolation and Separation of α - and β -Tubulin from Chick-Embryo Brain, Muscle and Skin

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Two kinds of tubulin, α and β , have been described in microtubules from many different systems. In the present study a new method is described for isolating and separating these two kinds of tubulin from chick-embryo brain, muscle and skin. The isolated tubulins were characterized by polyacrylamide-gel disc electrophoresis in the presence of urea and sodium dodecyl sulphate.

Tubulin is the main protein constituent of microtubules, which are involved in a number of physiological processes including cell division, cell motility, transport of proteins and support of extended structures (Gibbons, 1963; Hemminki, 1976).

Tubulin, the major constituent molecule of microtubules, is dimeric in NaCl solutions. It has a molecular weight of approx. 110000, but dissociates under denaturing conditions into polypeptide chains of molecular weight between 55000 and 60000 (Bryan, 1974; Bibring, 1976). Two non-identical chains (α - and β -tubulins) have been resolved by polyacrylamide-gel electrophoresis. The chains are usually found in equimolar amounts, regardless of the source of tubulin (Meza *et al.*, 1972). The present paper describes a new method for separating and isolating the α - and β -chains from each other.

Materials and Methods

All chemicals were purchased from Sigma (London) Chemical Co., Kingston upon Thames, Surrey, U.K., except Coomassie Blue, which was purchased from Serva, Heidelberg, Germany, and Sepharose, which was purchased from Pharmacia, Uppsala, Sweden.

Preparation of modified Sepharose

The modified Sepharose was prepared by the method of Hemminki (1976). Epoxy-activated Sepharose 6B containing a C₁₂ hydrocarbon spacer group (Pharmacia) was inactivated by incubating in 1M-ethanolamine, pH 8.0, overnight, followed by washes with 0.1M-sodium borate, pH 8.0, 0.1M-sodium acetate, pH 4.0, and 0.15M-NaCl.

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Preparation of tubulin

Tubulin was prepared from 13-day chick-embryo brain, muscle and skin by the method of Hemminki (1976). Chicken-embryo brains were homogenized in 30mM-sodium phosphate, pH 7.0, containing 10% (v/v) glycerol (about 10ml/brain) with 30 strokes of a Teflon/glass homogenizer at 4°C. The homogenate was centrifuged at 100000g for 60min, and the supernatant was used for further purification. A portion of the supernatant (6ml) was incubated with the modified Sepharose (1g). After incubation, the mixture of brain supernatant and modified Sepharose was centrifuged at 3000g for 10min at 4°C, and the supernatant was decanted. The modified Sepharose was put in a centrifuge tube and washed by centrifugation at 3000g for 10min with 30mM-sodium phosphate and then with 0.075M-NaCl solution at 4°C. Tubulin was eluted with 0.15M-NaCl, then precipitated in the supernatant with 50%-satd. (NH₄)₂SO₄, which was left at -20°C for 2h for complete precipitation. The precipitate was collected by centrifugation (at 3000g for 10min at 4°C).

Isolation and separation of α - and β -tubulins from each other

A new method is described below for isolating and separating α - and β -tubulins from each other. The tubulin was prepared from chick-embryo brain, muscle or skin as described above, and kept in 0.15M-NaCl solution. It was diluted to 0.075M-NaCl, then 2-mercaptoethanol added to a final concentration of 10% (v/v). The solution (2mg of tubulin/ml) was left at room temperature (20°C) for 10min, then incubated with modified Sepharose (6ml of tubulin solution/g of Sepharose). The unbound material (4.3mg) was collected and precipitated with 50%-satd. (NH₄)₂SO₄. The bound material (5.1mg)

was eluted from the Sepharose with 0.1 M-NaCl, then precipitated by 50%-satd. $(\text{NH}_4)_2\text{SO}_4$.

Urea/sodium dodecyl sulphate/polyacrylamide-gel disc electrophoresis

Tubulins were fractionated by a modification of the method of Bhorjee & Pederson (1973).

Preparation of polyacrylamide gels

'Discontinuous' gels [small-pore gel, 7.5% (w/v) acrylamide; large-pore gel, 2.25% (w/v) acrylamide], 8 cm in length, were prepared in glass tubes of internal diameter 0.5 cm. The lower (small-pore) gel was prepared by mixing 20 ml of 0.2 M-sodium phosphate buffer, pH 7.0 (containing 10 mM-EDTA and 0.2% sodium dodecyl sulphate), 3 ml of 8 M-urea, 10 ml of acrylamide solution (30%, w/v, acrylamide containing 0.85% *NN*-dimethylethylenediamine), 1.5 ml of ammonium persulphate solution (15 mg/ml) and 50 μ l of *NNN'*-tetramethylethylenediamine, then adjusted to 40 ml with water. The lower-gel mixture was poured into the glass tubes; after the gel hardened, the water was replaced by upper (large-pore)-gel mixture. The large-pore gel was prepared by mixing 8 ml of 0.02 M-sodium phosphate buffer, pH 6.0, 0.5 ml of 8 M-urea, 0.75 ml of acrylamide solution, 0.1 ml of 10% (w/v) sodium dodecyl sulphate, 0.4 ml of ammonium persulphate solution (15 mg/ml) and 10 μ l of *NNN'*-tetramethylethylenediamine, then adjusted to 10 ml with water. The gels began to set in a few minutes, but were left for 1 h at room temperature before use.

Electrophoresis was carried out at room temperature in 0.1 M-sodium phosphate buffer, pH 7.0 (in 5 mM-EDTA, pH 7.0, and 0.1% sodium dodecyl sulphate), as running buffer. The gels were pre-run for about 30 min before the tubulin sample was applied. Gels were run at 5 mA/gel (8 cm long $\times$ 0.5 cm diam.). Tubulin (1 mg/ml) was dissolved in 0.01 M-sodium phosphate buffer, pH 7.0 [containing 0.2% sodium dodecyl sulphate, 2% (v/v) 2-mercaptoethanol, 5 mM-EDTA and Bromophenol Blue], then denatured by heating at 70°C for 15 min. Tubulin solutions (30 μ l) were layered on the gels with a micro-pipette and without removing the buffer. Electrophoresis was then continued at the same current for up to 6 h. After the run the gels were blown out of the tubes by pushing water with a rubber teat. Gels were stained in 0.1% Coomassie Blue in 7% (v/v) acetic acid overnight, and destained in 7% acetic acid (in 10%, v/v, diethylene glycol). Gels were scanned at 585 nm after being placed in Perspex tubes of similar diameter.

Fig. 1. Urea/sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of tubulins

(a) Chick-embryo brain tubulin [bovine serum albumin used as a molecular-weight (68 000) standard]; (b) the bound material (α -tubulin); (c) the unbound material (β -tubulin); (d) mixture of bound and unbound materials (α - and β -tubulins).

Results and Discussion

When tubulins were extracted from 13-day-old chick-embryo brain, muscle or skin, solubilized in phosphate buffer, pH 7.0, containing 3% (v/v) 2-mercaptoethanol, and then subjected to electrophoresis on 7.5% (w/v) 'discontinuous' polyacrylamide gels containing urea and sodium dodecyl sulphate, two bands were obtained (α - and β -tubulins) (Fig. 1a). The molecular weights of those two polypeptide chains (α - and β -tubulins) were about

55000, with bovine serum albumin (mol.wt. 68000) as standard.

A major obstacle to analysing the differences between the two forms of tubulin and between tubulins from different sources has been the difficulty in separating and isolating them. In this new method, 2-mercaptoethanol was used to dissociate α -tubulin from β -tubulin; these were then incubated with the modified Sepharose at 0.075M-NaCl (pH 7.0) for 10 min. The binding was sensitive to ions and the bound material (after dissociation with mercaptoethanol) was eluted at 0.15M-NaCl. The unbound and the bound materials were characterized by electrophoresis on 'discontinuous' polyacrylamide gels (7.5%) containing urea and sodium dodecyl sulphate as described above. Figs. 1(b) and 1(c) show that the unbound material is α -tubulin and the bound material is β -tubulin, and each one is free from the other. Because the binding is sensitive to ions, α -tubulin does not bind to the modified Sepharose at 0.075M-NaCl, but β -tubulin does. After the unbound material and bound material were mixed together and subjected to electrophoresis, two bands, corresponding to α - and β -tubulins, appeared (Fig. 1d). β -Tubulin was the

faster-moving species in the gels and α -tubulin was the slower-moving one. The present system offers several distinct advantages for isolating and separating α - and β -tubulins.

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