A COMPARISON OF TUBULINS FROM MAMMALIAN BRAIN AND *PHYSARUM POLYCEPHALUM* USING SDS–POLYACRYLAMIDE GEL ELECTROPHORESIS AND PEPTIDE MAPPING

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Received 8 May 1980

1. Introduction

Tubulin isolated from mammalian brain consists of two equimolar protein species designated α- and β-tubulins. Conventionally α-tubulin is the slower, and β-tubulin the faster migrating species during sodium dodecyl sulphate–polyacrylamide gel electrophoresis [1]. Tubulin has been considered to be a highly conserved protein [2,3]; however, we now show differences between tubulins from mammalian brain and myxamoebae of the slime mould *Physarum polycephalum* from which microtubule proteins have been purified by an assembly procedure [4]. Evidence is based on differences in migration of these tubulins on SDS– and urea–SDS–PAGE, and peptide mapping, and demonstrates that *Physarum* tubulin consists of an α-tubulin which is similar to brain β-tubulin, and a β-tubulin which is dissimilar to both brain subunits. In addition, the analogous proteins from *Physarum* and brain demonstrate micro heterogeneity during urea–SDS–PAGE. The migration of the *Physarum* β-tubulin is altered during urea–SDS–PAGE such as to cause the order of migration of the myxamoebal tubulins to be reversed with respect to that during SDS–PAGE. In addition the importance of the source of SDS in resolving tubulin subunit proteins during PAGE is highlighted.

Abbreviations: EGTA, ethylene glycol-bis(β-aminoethyl ether) tetra acetic acid; PAGE, polyacrylamide gel electrophoresis; PIPES, piperazine-*N*,*N*′-bis(2-ethane-sulphonic acid); SDS, sodium dodecyl sulphate

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2. Experimental

Mammalian brain microtubule protein was prepared from fresh sheep brains by cycles of temperature-dependent assembly–disassembly as in [5]. Microtubule protein from myxamoebae of *Physarum polycephalum* (strain CLd) was prepared by assembly as in [4]. Tubulin from both sources was further purified by chromatography on phosphocellulose in 0.025 M PIPES, 0.5 mM EGTA, 0.25 mM $MgSO_4$, 0.1 mM GTP (pH 6.9). Phosphocellulose (Whatman P11) was prepared as in [6].

SDS–PAGE was performed according to [7] in gels of 6% (w/v) acrylamide with a 3% (w/v) acrylamide stacking gel, set in slabs 1 mm thick which facilitated rapid staining and destaining. For urea–SDS–PAGE, urea was present at 8 M in both main and stacking gels and at 6 M in the sample buffer. The urea–SDS gel solutions were filtered through a 0.45 μm Millipore membrane before polymerisation.

Protein samples were prepared for electrophoresis by dilution to the required concentration (0.1–1.0 mg/ml) in the appropriate sample buffer, followed by incubation at 100°C for 2 min before being cooled and loaded onto gels. Samples were electrophoresed for 2 h at a constant 12 mA. Gels were stained with Coomassie brilliant blue R250 in water:propan-2-ol: glacial acetic acid (65:25:10), destained in the above solvent mixture, and stored in 7% (v/v) acetic acid.

For experiments in which protein bands were excised, gels were stained for 15 min only and destained for 10 min to expose the bands sufficiently to enable accurate slicing of the gels. For photography, gels were stained for a minimum of 1 h. Protein bands excised from gels were re-equilibrated in

the appropriate sample buffer by incubation at room temperature for 45 min, with 3 changes of buffer, before being subjected to electrophoresis as above.

Peptide mapping of proteins in excised bands from SDS gels was done as in [8]. Proteolytic cleavage was achieved with 10 μl 0.1 mg/ml *Staphylococcus aureus* V8 protease (Miles Labs.). SDS was obtained from Fisons Scientific Apparatus, Loughborough, Leicester and from Sigma (London) Chem. Co., Poole. Acrylamide, *N,N'*-methylene bis-acrylamide (especially purified for electrophoresis), and Aristar grade urea were obtained from BDH Chemicals, Poole.

3. Results and discussion

It had been considered necessary to reduce and alkylate brain tubulin to resolve the two species on SDS gels [1]. It appears however, that this modification is unnecessary in the presence of a suitable brand of SDS. SDS from different suppliers was found to affect the migration of tubulin in SDS gels. This is illustrated in the case of sheep brain tubulin in fig.1. The use of SDS from Sigma in Laemmli SDS gels resulted in a good separation of brain α- and β-subunit proteins over a wide range of protein loading, whereas, in the presence of SDS from Fisons, the two tubulin species are incompletely separated. Similar observations have been briefly reported in [9] where SDS from different suppliers gave rise to variability in the migration of reduced– carboxymethylated sea urchin tubulin. This variability was attributed to impurities in the SDS. Here we find that the Sigma SDS, which effects the best separation, is the less pure of the two types used. The nature of any impurities is not known.

The difference in behaviour of tubulins in the presence of SDS from different sources may be used to compare tubulins from mammalian brain and *Physarum polycephalum* myxamoebae. As shown in fig.2, SDS–PAGE in the presence of Sigma SDS, which results in a good resolution of brain tubulin (fig.2c), does not effect such a separation of *Physarum* tubulin (fig.2d). The reverse is true, however, in the presence of Fisons SDS (fig.2a,b). In this situation, the subunit proteins of tubulin are well resolved, whereas those of brain are not.

It appears that, during SDS–PAGE using either type of SDS, the β-tubulin of brain co-migrates with

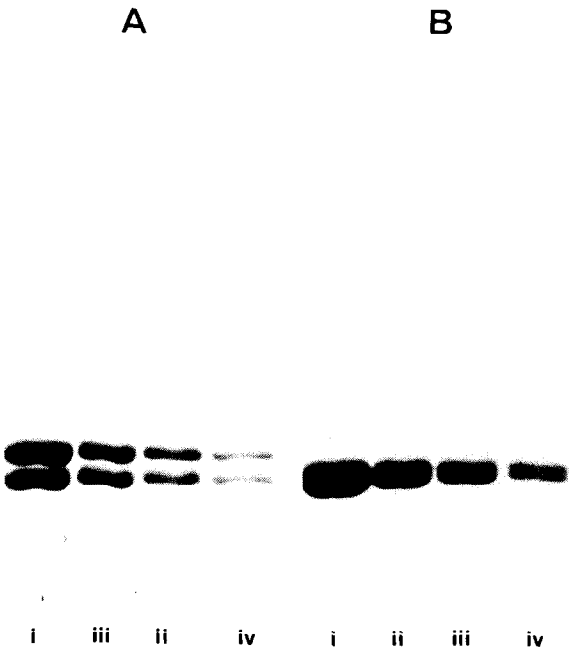


Fig.1. SDS–PAGE of sheep brain tubulin in 6% acrylamide gels containing: (A) Sigma SDS; (B) Fisons SDS. Protein loadings were for A and B: (i) 10 μg; (ii) 5 μg; (iii) 2.5 μg; (iv) 1.0 μg tubulin. Gels were stained with Coomassie brilliant blue.

the α-tubulin (slower migrating species) of *Physarum*.

The presence of urea in SDS gels, in addition to causing the retardation of tubulin migration in 6% acrylamide gels compared with gels containing SDS alone, also results in a change in the relative migration of α- and β-tubulins from both brain and *Physarum* myxamoebae. The pattern differs in both cases compared with that during corresponding SDS–PAGE. During urea–SDS–PAGE using Fisons SDS, both brain and *Physarum* tubulins are resolved into their subunits, and, in this system, the faster-migrating proteins from both sources appear to co-migrate (fig.2e,f). Similar results are obtained with Sigma SDS (fig.2g,h). In addition, the faster-migrating protein species of both brain and *Physarum* tubulin are resolved into multiple bands – two in *Physarum* and possibly three in brain.

In order to characterise further the behaviour of these tubulins during PAGE, tubulin subunits were separated on the SDS–PAGE system which gave the best resolution, i.e., using Sigma SDS for brain, and Fisons SDS for *Physarum* tubulin. Protein bands were then excised and subjected to electrophoresis on each

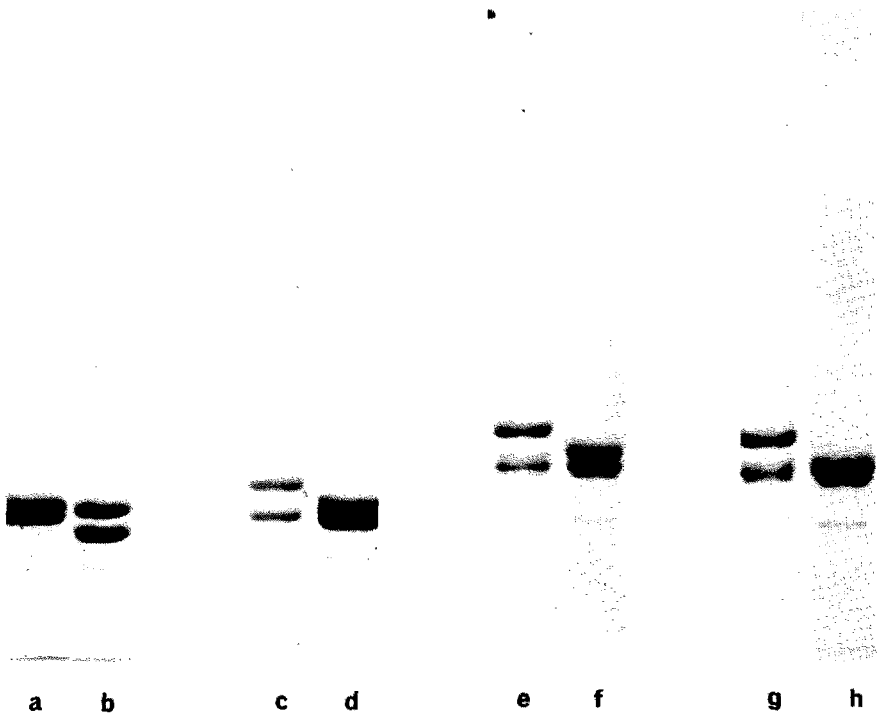
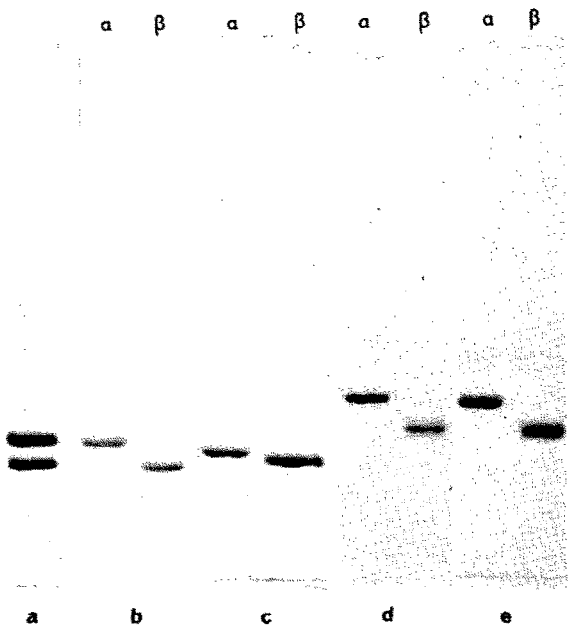


Fig.2. PAGE of sheep brain (a,c,e,g) and *Physarum* tubulin (b,d,f,h) on different gel systems. (a) Fisons SDS–PAGE; brain tubulin. (b) Fisons SDS–PAGE; *Physarum* tubulin. (c) Sigma SDS–PAGE; brain tubulin. (d) Sigma SDS–PAGE; *Physarum* tubulin. (e) Fisons urea–SDS–PAGE; brain tubulin. (f) Fisons urea–SDS–PAGE; *Physarum* tubulin. (g) Sigma urea–SDS–PAGE; brain tubulin. (h) Sigma urea–SDS–PAGE; *Physarum* tubulin.



of the four combinations of SDS–, and urea–SDS–PAGE systems. Results are shown in fig.3 for brain tubulin. The migration of the separate bands on re-electrophoresis was consistent with the pattern shown for 'whole' tubulin on each gel system as shown in fig.2. Results of identical manipulations with *Physarum* tubulin bands are shown in fig.4. As for brain, the pattern conformed to that shown in fig.2; when, however, excised bands were subjected to urea–SDS–PAGE the α- (slower migrating) band on SDS–PAGE became the faster migrating band, and vice versa for the SDS–PAGE β- (faster migrating) band. In

Fig.3. PAGE of excised protein bands of brain tubulin on different gel systems. (a) Tubulin separated by Sigma SDS–PAGE for excision of bands. (b) α and β bands re-run on Sigma SDS–PAGE. (c) α and β bands re-run on Fisons SDS–PAGE. (d) α and β bands re-run on Fisons urea–SDS–PAGE. (e) α and β bands re-run on Sigma urea–SDS–PAGE.

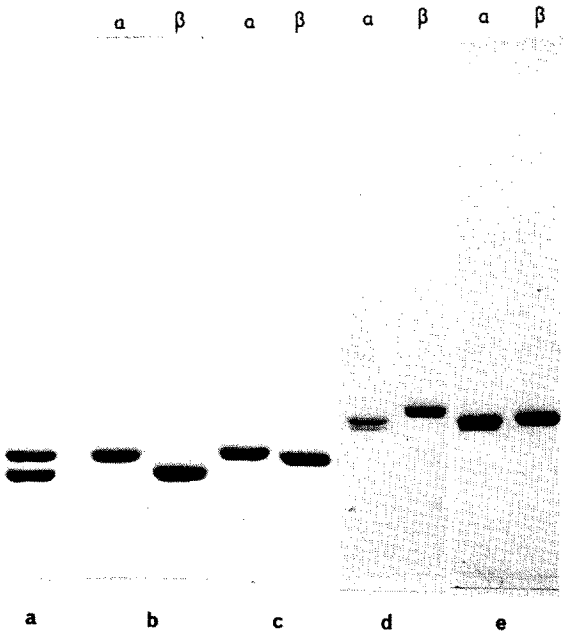


Fig.4. PAGE of excised protein bands of *Physarum* tubulin on different gel systems. (a) Tubulin separated by Fisons SDS–PAGE for excision of bands. (b) α and β bands re-run on Fisons SDS–PAGE. (c) α and β bands re-run on Sigma SDS–PAGE. (d) α and β bands re-run on Fisons urea–SDS–PAGE. (e) α and β bands re-run on Sigma urea–SDS–PAGE.

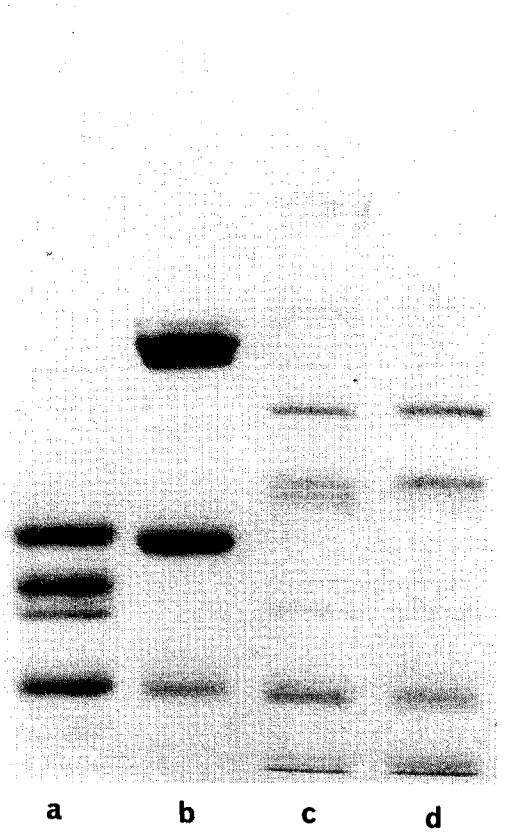


Fig.5. Peptide maps of: (a) brain α-tubulin; (b) *Physarum* β-tubulin; (c) *Physarum* α-tubulin; (d) brain β-tubulin. Acrylamide gel (15%) stained with Coomassie brilliant blue.

other words, in the presence of urea during SDS–PAGE, the positions of the tubulin species from *Physarum* myxamoebae are apparently reversed compared with their migration during SDS–PAGE. These results were independent of the source of SDS used. Identical results were obtained using gels in which the urea was pre-treated with Amberlite resin to remove thiocyanate and inorganic salts.

The apparent reversal of the *Physarum* tubulin migrations is due to an alteration in the behaviour of one of the tubulin bands (the faster moving species in SDS, which becomes the trailing band in the presence of SDS and urea). Such anomalous behaviour may be the result of decreased SDS binding by this protein in the presence of urea. Such a reduction has been reported for some other proteins [16].

These experiments suggest an analogy between the β-tubulin of brain and the α-tubulin of *Physarum* myxamoebae. This is substantiated by evidence from peptide mapping (fig.5) which shows that the pattern of peptides produced by *Staphylococcus aureus* V8 protease digestion of brain β- and *Physarum* α-tubulins is virtually identical. Maps of brain α-tubulin and *Physarum* β-tubulin are dissimilar.

Further evidence of the similarity between these two proteins is the fact that both demonstrate microheterogeneity within the subunit protein. This is seen most clearly on urea–SDS–PAGE (fig.3A(iii,iv), 3B(iii,iv)). Heterogeneity of both α- and β-tubulins has been demonstrated by isoelectric focussing on polyacrylamide gels [10], by two-dimensional gel electrophoresis in brain [11], and in *Aspergillus* [12]. A second β-tubulin subunit in brain has been shown by one-dimensional SDS–PAGE [13]. We present here further evidence in favour of the existence of multiple tubulin species. Tubulin from mammalian brain and *Physarum* myxamoebae therefore contain one protein in common. We propose to retain the conventional nomenclature of α- and β-tubulin for the slower and faster migrating species on SDS gels for

both brain and *Physarum* tubulin. With this convention the β-tubulin of brain is equivalent to the α-tubulin of *Physarum*. The β-tubulin of *Physarum* is dissimilar to both tubulin species from brain, and demonstrates anomalous behaviour during urea–SDS–PAGE. Various experiments including photoaffinity labeling have suggested that brain α-tubulin may be the subunit which binds colchicine [14]. Our demonstration that *Physarum* tubulin does not contain a protein analogous to brain α-tubulin may explain the failure of *Physarum* tubulin to bind colchicine [4,15] and the consequent insensitivity of this organism to the drug. In addition this is further evidence towards confirming that the brain α-tubulin may be the site of colchicine binding.

Differences between higher and lower eukaryote tubulins therefore do exist, confirming the indirect evidence suggested by differences in apparent drug sensitivity and binding. This work also highlights the advantages of using a variety of PAGE systems, and especially peptide mapping to establish the homology, or otherwise, of tubulin from different sources.

Acknowledgements

We thank Mrs M. Wilcox for excellent technical assistance, also the Canterbury abattoir for supplying sheep brains. This work was supported by the Science Research Council and the Wellcome Trust.

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