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Complete amino acid sequence of β-tubulin from porcine brain

(sequence microheterogeneity/similarity to α-tubulin, troponin T, and nucleotide binding proteins)

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ABSTRACT The primary structure of porcine brain β-tubulin was determined by automated and manual Edman degradation of six sets of overlapping peptides. The protein consists of 445 amino acid residues and has a minimum of six positions that are heterogeneous, indicating at least two β-tubulins in porcine brain. Comparison of the optimally aligned sequences of α-tubulin and β-tubulin indicates that 41% of their primary structures are identical. A region rich in glycyl residues is similar both in sequence and predicted secondary structure to the phosphate binding loop of several nucleotide binding enzymes. β-Tubulin contains a highly acidic COOH-terminal region that resembles the NH_2-terminus of troponin T.

Microtubules are helical arrays of alternating globular units of α-tubulin and β-tubulin, each having a molecular weight of 50,000, a similar amino acid composition, and a similar overall shape (for review, see ref. 1). Yet these subunits seem to differ in function, as indicated by the binding of exchangeable GTP to β-tubulin (2) and by the participation of a tissue-specific β-tubulin in the meiotic spindle and other stages of spermatogenesis in *Drosophila* (3, 4).

β-Tubulin mutants have been shown to cause reduced binding of cytostatic drugs (5, 6) and were used to demonstrate the involvement of microtubules in nuclear movement in germinating spores of *Aspergillus* (7). A knowledge of the primary structure of β-tubulin, along with that of α-tubulin, could be helpful in understanding how different tubulins function, how the assembly of microtubules into various organelles is regulated, and how certain drugs and nucleotides are bound with high affinity. The sequence of α-tubulin has already shown several variants of this protein in brain (8). Regional similarities were found to muscle proteins, indicating the possibility of common biochemical features in muscular and microtubular movement. Here we present the sequence of β-tubulin from porcine brain and compare it with that of the α-chain.

MATERIALS AND METHODS

Tubulin was purified from porcine brain as described (8). The 100,000 × *g* brain supernatant in 0.05 M sodium pyrophosphate buffer, pH 7.0, was chromatographed on DEAE-cellulose with a linear gradient of 0.1–0.3 M sodium chloride. The protein was reduced and alkylated with iodoacetic acid and assayed for contaminating proteins by disc gel electrophoresis in the system of Yang and Criddle (9) using 8% polyacrylamide gels. α- and β-chains were separated on hydroxylapatite in 0.1% $NaDodSO_4$ with a linear gradient of 0.2–0.4 M sodium phosphate (10). Fractions were assayed for purity by gel electrophoresis as above. Only β-chain preparations containing <5% impurities were used for sequence determination.

To remove $NaDodSO_4$, the protein was dialyzed against 1 mM ammonium bicarbonate, then, the solution was concentrated by vacuum evaporation and brought to pH 5.5 with acetic acid, and the protein was precipitated with 9 vol of ice-cold acetone. The supernatant was discarded after 2 hr at −20°C, and the precipitate was dissolved in dilute ammonium hydroxide and dialyzed against 0.01 M ammonium bicarbonate. Enzymatic digestion was done at pH 8.0 with 1–4 mg of β-tubulin per ml and generally at an enzyme/substrate ratio of 1:100 and 37°C. Fifty milligrams of β-tubulin was digested with either thrombin (Sigma), affinity-purified trypsin (a gift from K.-D. Jany, Stuttgart) (11), chymotrypsin (Merck), or protease from *Staphylococcus aureus* (Miles) or from *Astacus fluviatilis* (donated by R. Zwilling, Heidelberg; ref. 12). Digestions with staphylococcal protease were done in 0.1 M ammonium bicarbonate for 24 hr and those with astacus protease (enzyme/substrate = 1:50) were done at 20°C in 0.1 M ammonium bicarbonate for 4 hr. Cleavage times with chymotrypsin were 4.5 hr and those with trypsin and thrombin were 7 hr.

Acetone-precipitated β-tubulin was digested with cyanogen bromide by dissolving the protein in pure formic acid, diluting the solution to 70% acid, and adding CNBr (150-fold excess over methionyl residues). After 24 hr in the dark, the reaction mixture was lyophilized.

Digests of β-tubulin were fractionated on Sephadex G-50 and G-100 in 8 M urea/0.1 M ammonium bicarbonate, and pooled material was desalted on Sephadex G-10. Peptides were further separated by chromatography on DEAE-cellulose, Dowex 1 × 2 and 50 × 2, cellulose thin layers and, more recently, by reversed-phase high-pressure liquid chromatography on a Zorbax C-8 column with a Du Pont 850 liquid chromatograph, using 0.05 M ammonium bicarbonate brought to pH 7.5 with acetic acid and gradients of 0–60% acetonitrile at 40°C (13).

Amino acid analyses were performed on a Durrum D-500 analyzer. Automated Edman degradations on a Beckman 890 C sequencer were done by using a single cleavage program (14) with 0.1 M quadrol as a buffer. To reduce peptide losses due to solvent extraction, 3 mg of Polybrene and 200 μg of Gly-Gly were applied to the cup and subjected to three cycles of degradation prior to analyzing the sample (15). Phenylthiohydantoin derivatives of amino acids were identified by high-pressure liquid chromatography (16).

RESULTS AND DISCUSSION

The presence of soluble peptide aggregates in digested material initially impeded progress in determining the primary structure of β-tubulin. The addition of 8 M urea in most purification steps eliminated this problem but resulted in the loss of small and

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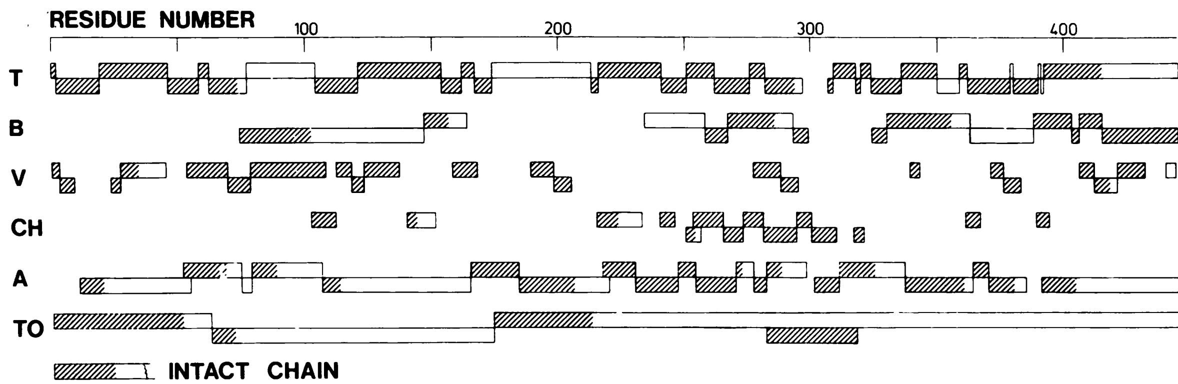

FIG. 1. Fragments generated in the sequence analysis of β-tubulin. The hatched section of each bar indicates the portion of the sequence determined. Peptides were generated by trypsin (T), cyanogen bromide (B), protease from *Staphylococcus aureus* V8, chymotrypsin, protease from astacus, and thrombin.

insoluble peptides on desalting and partial blockage of amino groups by cyanate from the decomposing urea. The application of high-pressure liquid chromatography techniques, however, made the use of urea unnecessary and the high resolution enabled larger amounts of pure fragments to be obtained in fewer steps. This resulted in a greatly increased rate of sequence analysis progress, which is reflected in Fig. 1. The tryptic and astacus peptides, separated by high-pressure liquid chromatography, contributed larger amounts of sequence data than the cyanogen bromide and chymotryptic fragments, which were isolated in the presence of urea. The astacus protease is a new aid in protein sequencing; we found it to cleave preferentially to the NH_2-terminal side of small residues. Bonds at about one-third of the alanines, one-fourth of the serines and threonines, and one-seventh of the glycines present in β-tubulin were cleaved.

The complete sequence of the tubulin β-chain, comprising 445 amino acid residues, is shown in Fig. 2. The COOH-terminal 45 residues are a particularly acidic region of the chain. Five aspartate and 14 glutamate residues account for 42% of the positions, and the remainder are devoid of basic amino acid side chains, as well as of cysteine, isoleucine, and proline. In contrast, glutamate, the most frequent amino acid of β-tubulin, is absent over a length of 82 residues between positions 206 and 287 and, whereas two-thirds of positions 140–148 are glycine residues, the following 74 residues contain none at all. A similar imbalance is observed for methionine: only 4 are found in the NH_2-terminal half, but 14 are found in the COOH-terminal half.

As it has frequently been claimed that tubulin is associated with membranes, we used the method of Segrest and Feldmann (17) to evaluate the hydrophobicity of different regions. Resi-

FIG. 2. Amino acid sequence of β-tubulin from porcine brain. Positions 37, 48, 57–59, and 275 are heterogeneous.

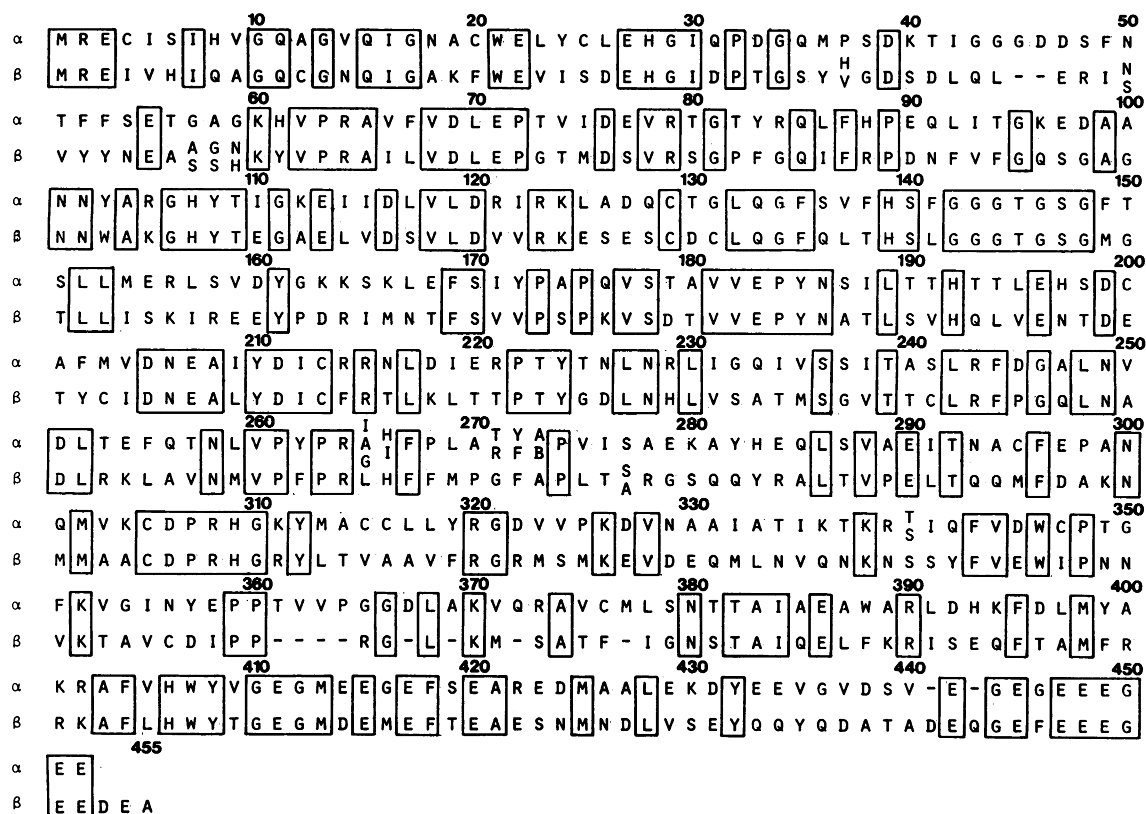

FIG. 3. Comparison of α- and β-tubulin from porcine brain. Boxes indicate identical residues. Gaps were introduced at positions 441 and 443 in α-tubulin and 45–46, 361–364, 367, 369, 372, and 377 in β-tubulin, respectively, to obtain maximal homology. The numbering of residues is therefore different from that in Fig. 2.

dues 253–261 (index 2.16) and 263–274 were the most hydrophobic (index 2.50; the scale is based on the free energy of transfer of amino acids from ethanol to water with alanine arbitrarily set at 1.0 and glycine set at zero). Thus, this region appears to be the most likely candidate for hydrophobic interaction.

We did not observe any posttranslational amino acid modifications of β-tubulin. A phosphorylated tryptic peptide of rat brain tubulin, however, isolated by Eipper (18) after *in vivo* ^{32}P labeling, has almost exactly the composition of the COOH-terminal tryptic peptide, residues 393–445, of porcine brain β-tubulin. As the phosphorylated residue is a serine (19), it should be either serine 413 or serine 420. In agreement with these data, both of these positions are predicted to be in a β-turn and thus on the surface of the polypeptide chain.

Microheterogeneity. At least six positions (37, 48, 55–57, and 275) are heterogeneous. The tryptic peptide comprising residues 47–58 was isolated in roughly equimolar amounts in two fractions having different amino acid compositions. The two forms were degraded separately and yielded the sequences Ile-Asn-Val-Tyr-Tyr-Asn-Glu-Ala-Ala-Gly-Asn-Lys and Ile-Ser-Val-Tyr-Tyr-Asn-Glu-Ala-Ser-Ser-His-Lys, respectively. Similarly, the heterogeneity in positions 37 and 275 was determined with separate homogeneous peptide fractions. This indicates the presence of at least two slightly differing β-tubulins in porcine brain. All of the exchanges except histidine for valine in position 37 can be achieved by single-base substitutions in their respective codons. This heterogeneity might be due to alleles or to different cell types of the brain, such as nerve and glia. The presence of more than one β-tubulin specific for different cells in one organism has been established by Kemphues *et al.* (3, 4) for the ovaries and the testes of *Drosophila*. Alternatively, more than one type of tubulin may be required for the assembly of microtubules having different functions in one cell.

Secondary Structure Prediction. As calculated by the method of Chou and Fasman (20), the major helix potentials reside in the COOH-terminal half of the chain, residues 245–254, 288–302, 321–331, 373–393, 401–412, and 426–445. A prominent β-sheet with seven strands is expected at positions 163–242, and a series of overlapping turns is predicted at positions 26–42, 54–59, 69–82, 93–101, 137–149, 304–310, and 335–341. Except for residue 48, all heterogeneous positions are expected to be located in turns and hence at the surface of the protein. It is predicted that 24% of the chain is in a helical conformation and 29% is in a β-sheet conformation. These predictions, together with those for α-tubulin (8), are fairly consistent with the results of circular dichroism studies on native tubulin (21, 22).

Pig Brain β- and α-Tubulin. A comparison of pig brain β- and α-tubulins (8) shows 41% to 42% sequence identity, depending on the variant (Fig. 3). This structural similarity is sufficient to indicate a common ancestral gene for both chains. It would appear that the divergence of α- and β-tubulin has brought about some functional specialization, as indicated, for example, by the preferential binding of exchangeable GTP to β-tubulin (2). As the overall shape of α- and β-tubulin in the microtubule appears to be similar (1), the conserved parts of the structure may be responsible for shaping the building blocks of microtubules, while the dissimilar regions may be important for specialized functions such as subunit interactions and binding of ligands or other proteins. There is a large region of dissimilarity at residues 313–379, and smaller ones are found at positions 35–59, 153–168, 265–285, and 422–441. They comprise all the heterogeneous positions, as well as the insertions introduced to achieve maximum homology. The heterogeneous

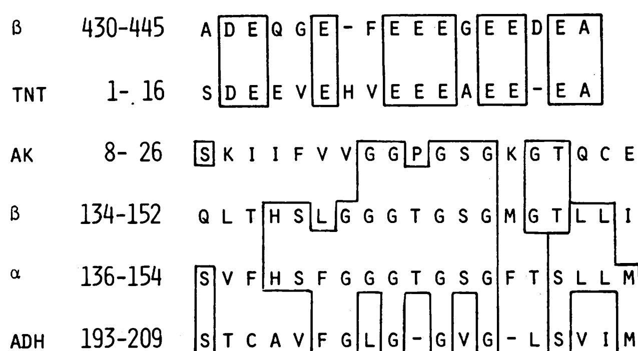

FIG. 4. Homology of β-tubulin (β) with troponin T (TNT) from rabbit skeletal muscle (27), adenylate kinase (AK) from porcine muscle (28, 29), α-tubulin (α) (8), and alcohol dehydrogenase (ADH) from horse (30, 31).

positions differ considerably in both chains, in α-tubulin they occur mainly around position 270 and in the β-chain they occur around position 50.

Of the 12 cysteine residues in α-tubulin and the 8 in the β-tubulin, only three in each subunit are found in identical positions (129, 213, 305). This may reflect the absence of disulfide bridges, as estimates of disulfides in tubulin vary between zero (18) and one per subunit (23) in different sources. The modification of only one cysteine per tubulin subunit has been shown to prevent polymerization (24, 25), indicating a possible role for this residue in microtubule assembly.

The longest conserved sequence, seven residues in positions 142–148, contains a cluster of glycines. It may represent a flexible loop involved in phosphate binding.

Similarity to Other Tubulins. Comparison with data on the NH_2-terminal 25 residues of chicken brain β-tubulin and β-tubulin from sea urchin sperm outer doublets (26) shows four and at least five amino acid substitutions, respectively. Residues 10, 13, and 17 have been identified as threonine in chicken brain and sea urchin and glycine in porcine brain. Cysteine is present in position 12 in β-tubulin, which has been reported tentatively as serine in the chicken and sea urchin protein; lysine in position 19 of porcine brain β-tubulin is an unidentified residue in the other two sources.

Similarity to Other Proteins. As shown in Fig. 4, the COOH-terminal 15 residues of β-tubulin resemble the NH_2-terminus of troponin T. Nine of the 10 acidic residues present are conserved. A very high helical potential ($P_\alpha = 1.40$) is predicted for both sequences, suggesting that they may serve similar functions (e.g., binding of cationic proteins).

A conspicuous feature in both tubulin chains is the glycine cluster of residues 142–148 (Fig. 3). Despite its lack of side chains, it is the longest conserved sequence and was recognized by Schulz as being similar to the region binding the phosphate groups of ATP in pig muscle adenylate kinase (28, 29). Similar nucleotide binding folds have been shown to be a common feature of dehydrogenases, kinases, and flavodoxins (32). They comprise a β-sheet strand, followed by a flexible loop terminated by a glycine, and an α-helix. This region is summarized in Fig. 4 for the tubulin chains, adenylate kinase, and alcohol dehydrogenase (30, 31). The spatial structure of this region is known from x-ray diffraction analysis of the kinase and the dehydrogenase and is compatible with the predicted secondary structure of β- and α-tubulin. The glycine clusters of both tubulin subunits may therefore be the binding sites for phosphate groups of the exchangeable and nonexchangeable GTP (33).

Other similarities, not shown here, including the predicted conformation, exist between regions of β-tubulin and actin. On the whole, however, tubulins constitute a new protein family unrelated to other known proteins.

Elucidation of the amino acid sequences of both tubulin subunits from one source should facilitate investigations on the functions of various regions of the tubulin molecule. It will also help to answer questions regarding the existence of separate tubulins in different organs, the molecular basis of functional defects in tubulin mutants, and the tertiary structure of the whole protein and its binding sites for cytostatic agents.

After this paper was submitted for review, the nucleotide sequence of cDNA from chicken brain tubulin messengers was published (34). In the deduced tubulin sequence about 10% of the α-chain is missing. Seven residues differ from the sequence given in Fig. 3: α—arginine-175, tyrosine-295, and glutamate-358; β—threonine-57, methionine-172, serine-298, and isoleucine-318. The evolutionary mutation rate appears to be comparable with that of histones.

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1. Amos, L. A. (1979) in *Microtubules*, eds. Roberts, K. & Hyams, J. S. (Academic, New York), pp. 1–64.
2. Geahlen, R. L. & Haley, B. E. (1979) *J. Biol. Chem.* **254**, 11982–11987.
3. Kemphues, K. J., Raff, R. A., Kaufman, T. C. & Raff, E. C. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 3991–3995.
4. Kemphues, K. J., Raff, E. C., Raff, R. A. & Kaufman, T. C. (1980) *Cell* **21**, 445–451.
5. Sheir-Neiss, G., Lai, M. H. & Morris, N. R. (1978) *Cell* **15**, 639–647.
6. Cabral, F., Sobel, M. E. & Gottesman, M. M. (1980) *Cell* **20**, 29–36.
7. Oakley, B. R. & Morris, N. R. (1980) *Cell* **19**, 255–262.
8. Ponstingl, H., Krauhs, E., Little, M. & Kempf, T. (1981) *Proc. Natl. Acad. Sci. USA*, in press.
9. Yang, S. & Criddle, R. S. (1970) *Biochemistry* **9**, 3063–3072.
10. Little, M. (1979) *FEBS Lett.* **108**, 283–286.
11. Jany, K. D., Keil, W., Meyer, H. & Kiltz, H. H. (1976) *Biochim. Biophys. Acta* **453**, 62–66.
12. Sonneborn, H. H., Zwilling, R. & Pfleiderer, G. (1969) *Hoppe-Seyler's Z. Physiol. Chem.* **350**, 1097–1102.
13. Ponstingl, H., Krauhs, E., Little, M., Kempf, T., Hofer-Warbinek, R. & Ade, W. (1981) in *High Pressure Liquid Chromatography in Protein and Peptide Chemistry*, eds. Lottspeich, F., Henschen, A. & Hupe, K. P. (de Gruyter, Berlin), in press.
14. Brauer, A. W., Margolies, M. N. & Haber, E. (1975) Biochemistry **14**, 3029–3035.
15. Tarr, G. E., Beecher, J. F., Bell, M. & McKean, D. J. (1978) *Anal. Biochem.* **84**, 622–627.
16. Zimmerman, C., L., Appella, E. & Pisano, J. J. (1977) *Anal. Biochem.* **77**, 569–573.
17. Segrest, J. P. & Feldmann, R. J. (1974) *J. Mol. Biol.* **87**, 853–858.
18. Eipper, B. A. (1974) *J. Biol. Chem.* **249**, 1407–1416.
19. Eipper, B. A. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 2283–2287.
20. Chou, P. Y. & Fasman, G. D. (1978) *Annu. Rev. Biochem.* **47**, 251–276.
21. Ventilla, M., Cantor, C. R. & Shelanski, M. (1972) *Biochemistry* **11**, 1554–1561.
22. Lee, J. C., Corfman, D., Frigon, R. P. & Timasheff, S. N. (1978) *Arch. Biochem. Biophys.* **185**, 4–14.
23. Lee, J. C., Frigon, R. P. & Timasheff, S. N. (1973) *J. Biol. Chem.* **248**, 7253–7262.
24. Kuriyama, R. & Sakai, H. (1974) *J. Biochem. (Tokyo)* **76**, 651–654.
25. Ikeda, Y. & Steiner, M. (1978) *Biochemistry* **17**, 3454–3459.
26. Luduena, R. F. & Woodward, D. O. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 3594–3598.
27. Pearlstone, J. R., Johnson, P., Carpenter, M. R. & Smillie, L. B. (1977) *J. Biol. Chem.* **252**, 983–989.

28. Schulz, G. E., Elzinga, M., Marx, F. & Schirmer, R. H. (1974) *Nature (London)* **250,** 120–123.
29. Pai, E. F., Sachsenheimer, W., Schirmer, R. H. & Schulz, G. E. (1977) *J. Mol. Biol.* **114,** 37–45.
30. Jörnvall, H. (1970) *Eur. J. Biochem.* **16,** 25–40.
31. Ohlsson, I., Nordström, B. & Brändeń, C.-I. (1974) *J. Mol. Biol.* **89,** 339–354.
32. Rossmann, M. G. & Argos, P. (1977) *J. Mol. Biol.* **109,** 99–129.
33. Weisenberg, R. C., Borisy, G. G. & Taylor, E. W. (1968) *Biochemistry* **7,** 4466–4479.
34. Valenzuela, P., Quiroga, M., Zaldivar, J., Rutter, W. J., Kirschner, M. W. & Cleveland, D. W. (1981) *Nature (London)* **289,** 650–655.