Interaction of Tubulin with Octyl Glucoside and Deoxycholate. 1. Binding and Hydrodynamic Studies†

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ABSTRACT: Tubulin purified from calf brain cytoplasm, normally a compact water-soluble dimer, is able to interact with the mild detergents octyl glucoside (a minimum of 60 detergent molecules) and deoxycholate (95 ± 8 molecules). Binding is cooperative and approaches saturation below the critical micelle concentration of the amphiphiles. Binding is accompanied by a quenching of the intrinsic protein fluorescence, but no spectral shape changes indicating denaturation such as in the case of sodium dodecyl sulfate are observed. Glycerol, which is known to be preferentially excluded from the tubulin domain and to favor the folded and associated forms of this protein, inhibits the binding of the mild detergents. Octyl glucoside induces a rapidly equilibrating tubulin self-association reaction characterized by a bimodal sedimentation velocity profile with boundaries at approximately 5 and 12 S. Full dissociation of this detergent restores the normal sedimentation behavior to 90% of the protein. Binding of deoxycholate slows the sedimentation velocity of tubulin from $s^0_{20,w}$ = 5.6 ± 0.2 S to $s^0_{20,w}$ = 4.8 ± 0.3 S. Measurements of the molecular weight of the tubulin–deoxycholate complex indicate an increase from 100 000 to 143 000 ± 5000. The diffusion rate consistently decreases from (5.3 ± 0.5) × 10^{-7} to (3.8 ± 0.2) × 10^{-7} cm^2 s^{-1}. This is most simply interpreted as an expansion of the undissociated tubulin dimer upon detergent binding (a change in the frictional ratio, f/f_{min}, from 1.35 to 1.86). It is concluded that tubulin shows a reversible transition between the water-soluble state and amphipathic detergent-bound forms which constitute a model system of tubulin–membrane interactions.

Tubulin is the major component of cytoplasmic, flagellar, and ciliar microtubules. However, forms of this protein have been reported to be associated to other cellular structures. Postsynaptic densities were found to contain a substantial amount of tubulin on the basis of immunohistochemistry (Walters & Matus, 1975), two-dimensional gel electrophoresis, and tryptic mapping (Kelly & Cotman, 1978). More recently tubulin has been recognized as a component of brain and liver coated vesicles (Pfeffer et al., 1983; Kelly et al., 1983; Wiedenmann & Mimms, 1983). Except for these cases of tight, detergent-resistant association of tubulin to cellular structures, most other reports of nonmicrotubular proteins related to tubulin indicate association to cell membranes. Battacharyya and Wolff (1975) reported that a substantial portion of the [^{3}H]colchicine binding activity in brain and thyroid tissue homogenates was associated to particulate cell fractions and could be released by nonionic detergents. In this case, the very specific enhancement of colchicine fluorescence characteristic of its binding to tubulin was not detected. Tubulin-like proteins have been identified in the plasma membrane of lymphoid cells (Bachvaroff et al., 1980; Rubin et al., 1980; Quillen et al., 1985), platelet membranes (Steiner, 1983), brain myelin (Reig et al., 1982; de Nêchaud et al., 1983) and brain mitochondria (Hargreaves & Avila, 1985).

Zisapel et al. (1980) reported that α-tubulin was an integral protein of synaptic vesicle membranes, requiring ionic detergents for solubilization, while the β-tubulin content of these membranes depended on the presence of divalent cations in the buffer, suggesting a more peripheral attachment. Membrane proteins similar to tubulin from molluscan gill ciliary membranes (Stephens, 1977, 1981, 1983) and *Tetrahymena* ciliary membranes (Dentler, 1980; Dentler et al., 1980) are more extensively characterized. Neutral detergent solubilization of the membranes of scallop cilia released [^{3}H]colchicine binding activity and a major protein of apparent M_r 55 000 that could be resolved into α and β components (Stephens, 1977). These were coincident in two-dimensional gel electrophoresis with α- and β-tubulin from the B subfiber of cilia and could be distinguished in systems of polyacrylamide gel electrophoresis sensitive to differences in dodecyl sulfate binding. Comparative tryptic peptide mapping revealed a 90% coincidence of peptides and two to three prominent peptides exclusive of the membrane or axonemal subunits. Charge shift electrophoresis showed that membrane tubulin was able to bind more anionic and more cationic detergent than axonemal tubulin. These chemical differences argued against any artifactual origin of the detergent-solubilized tubulin by simple breakdown of the axoneme and were interpreted as due to the presence of a distinct membrane tubulin isotype (Stephens, 1981). Interestingly, the proteins of the membrane extract remained soluble upon detergent removal; freezing and thawing resulted in the formation of protein–lipid vesicles whose composition closely resembles that of the original material. Exogenous tubulin was not appreciably incorporated into the reconstituted material, and membrane tubulin was resistant to extraction with chaotropic agents (Stephens, 1983).

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All these studies clearly point to the existence of integral membrane proteins closely related to tubulin. These are commonly called membrane tubulins. Such proteins could be involved in the association of organelle membranes to microtubules or constitute membrane receptors for small molecules. The primary structure and binding activities of these proteins are not known. A crucial question is whether membrane tubulins are the result of posttranslational modification of cytoplasmic tubulin or whether, on the other hand, they could be products of homologous genes. Alternatively, it may be asked whether cytoplasmic tubulin can exhibit hydrophobic interactions and be incorporated into membranes. Such a property would not necessarily result from a physiological situation but could be a reflection of the general properties of all tubulins and constitute a useful model system. Since, in contrast to membrane tubulin, mammalian cytoplasmic brain tubulin is at present an extensively characterized protein (Lee, 1983; Na & Timasheff, 1982), it is worthwhile addressing this question. The assembly of brain microtubule protein was reportedly inhibited by hepatic membrane fractions and liposomes prepared from membrane-extracted phospholipids (Reaven & Azhar, 1981). Simonin et al. (1981) reported that tubulin binds to plasmacytoma membranes and stimulates its basal adenylate cyclase activity. Caron and Berlin (1979) reported the adsorption of microtubule proteins to unilamellar dimyristoylphosphatidylcholine vesicles, leading to the formation of multilamellar structures. Klausner et al. (1981) examined the interaction of phosphocellulose-purified tubulin from bovine brain with unilamellar phospholipid vesicles. An interaction was found at the phase transition temperature of dipalmitoylphosphatidylcholine resulting in the release of carboxyfluorescein from the vesicles and the formation of stable tubulin–vesicle recombinants. The interaction was insensitive to ionic strength. Competition experiments indicated that once tubulin became incorporated into the vesicles, it was no longer available for interaction with further vesicles and that distearoyl- and dioleoylphosphatidylcholine vesicles were ineffective. On the other hand, Jouniau and DeCruyper (1986) have recently reported a predominantly ionic interaction of tubulin with dimyristoylphosphatidylcholine/dimyristoylphosphatidylglycerol vesicles, which is mediated by microtubule-associated proteins.

An established approach to the study of membrane and lipid-binding proteins is the measurement of their interactions with detergents, particularly with the nondenaturing amphiphiles which do not bind massively to common water-soluble proteins (Tanford, 1980). Possible large regions capable of hydrophobic interactions in soluble purified calf brain tubulin have been probed employing octyl glucoside and deoxycholate (Andreu, 1982). These detergents, one nonionic and one anionic, were selected because they do usually bind not to soluble proteins but to membrane proteins (Helenius et al., 1979) and also for their relatively high critical micelle concentrations and ease of removal and their availability in chemically homogeneous and labeled forms. The present work describes in detail the binding of these amphiphiles and their effects on the tubulin heterodimer association by means of fluorescence, equilibrium gel chromatography, and hydrodynamic techniques. The aim is to explore the cytoplasmic tubulin–mild detergent system as a simplified, rigorous solution model of tubulin–membrane interactions.

Materials and Methods

Protein, Ligands, and Other Materials. Calf brain tubulin was purified, stored in liquid nitrogen, and prepared for use, and its concentration was measured spectrophotometrically as described (Weisenberg et al., 1968; Lee et al., 1973; Andreu et al., 1984). Experiments were routinely performed in PG[1] buffer, pH 7.0.

Deoxycholic acid sodium salt (lot no. 710059) and octyl β-D-glucopyranoside (lot no. 110072 and 210131) were from Calbiochem. At neutral pH, deoxycholate is very close to its pK, and concentrated solutions tended to form a gel or precipitated (Helenius et al., 1979), particularly in the cold. This was avoided by keeping the solutions at 25 °C, not exceeding free detergent concentrations of approximately 6 mM, or, in certain experiments, operating at times before gelation occurred. [^{3}H]Deoxycholic acid (lot no. 1319-014 and 1336-148, 3–4 Ci/mol) and [*glucose*-^{14}C]-octyl-β-D-glucopyranoside (lot no. 1141-235, 0.3 Ci/mol) were from New England Nuclear. Their purity and stability were checked by thin-layer chromatography in silica gel sheets (Merck) developed with benzene–acetone–methanol (4:2:1) or benzene–dioxane–acetic acid (15:2:3). The batches employed were more than 95% radiochromatographically homogeneous.

GTP dilithium salt was from Boehringer-Mannheim. Colchicine was from Ega Chemie Co. Sephacryl S-300 and Sephadex G-25 were from Pharmacia. Glycerol was from Merck, analytical grade, and all other chemicals were of reagent grade.

Binding Measurements. Fluorescence spectra and measurements were made in a Fica MKII double-beam spectrofluorometer, employing 0.5 × 0.5 cm cells that were mounted on holders thermostated with a Lauda K2RD water bath. Due to the presence of slightly light-absorbing and fluorescent impurities in the detergents employed, two corrections were made in the measurements of protein fluorescence quenching by detergent binding. Inner filter effect was corrected by the graphical procedure of Mertens and Kägi (1979). As shown in Figure 2, this correction was typically less than 10% of the measurement. Fluorescence of detergent impurities was either compensated by the reference cuvette of the fluorometer or measured in controls without protein and subtracted. The latter correction only occasionally reached 20% of the measurement and was typically smaller than 5%. Once the corrected fluorescence, f, was obtained, the fractional change of protein fluorescence due to detergent binding was calculated as $\theta(f) = (f_c - f)/(f_c - f_m)$, where f_c is the fluorescence of the protein in the absence of detergent and f_m the fluorescence in the presence of excess detergent saturating the effect.

Direct binding measurements were made employing radiolabeled detergents by application of the Hummel and Dreyer (1962) equilibrium technique. Sephadex G-50 and Sephacryl S-300 (0.9 × 25) cm columns thermostated at 25 ± 0.5 °C were employed. Protein was determined spectrophotometrically and 0.5-mL aliquots of each fraction counted in a Beckmann L100 liquid scintillation spectrometer. Due to the large amount of nonbound detergent in comparison to the bound one, the changes in ligand concentration measured were small, and the method had to be carefully applied, as discussed elsewhere (Andreu, 1985). The molecular weight of the mammalian brain tubulin heterodimer is taken as 10^5 (Lee et al., 1973; Krauhs et al., 1981).

Fluorescence and column binding measurements were compared by two procedures, both relying on the assumption of $\theta(f)$ being equal to the fractional saturation, $\theta = \nu/n$. First, a linear regression analysis of ν on $\theta(f)$ was made that showed a proportional relation in the binding of deoxycholate (correlation coefficient 0.98) and indicated the number of binding

[1] Abbreviation: PG, 10 mM sodium phosphate and 0.1 mM GTP, pH 7.0.

sites, n, from the value of ν at $\theta(f) = 1$. Second, Hill plots (Hill, 1911) were constructed, plotting ln $[\theta/(1 - \theta)]$ vs. ln $[\text{ligand}]_{\text{free}}$, employing $\theta(f)$ or ν/n. For the binding of deoxycholate it proved possible to superimpose these.

The critical micelle concentrations of the detergent batches employed were measured by the increase in the fluorescence of anilinonaphthalenesulfonate (Eastman) (de Venditis et al., 1981) at pH 7.0 and 25 °C. The values for octyl glucoside in PG and the same buffer containing 3.4 M glycerol were 24 ± 1 and 23 ± 1 mM, respectively; the corresponding values for deoxycholate were 4.8 ± 0.2 and 5.6 ± 0.2 mM.

Analytical Ultracentrifugation. A Beckman Model E centrifuge, equipped with electronic speed control, RTIC unit, and photoelectric scanner, was employed.

Sedimentation velocity experiments were made at 50 000 rpm and 20 or 25 °C. In runs employing the photoelectric scanner three samples were loaded in double sector cells in an AnF rotor. In experiments employing schlieren optics, two samples were loaded in double sector cells with regular and wedge windows in an AnD rotor. Profiles were recorded on Kodak metallographic plates, and the position of the maxima was measured in a Nikon microcomparator equipped with digital display. Relative viscosities of detergent solutions identical with those employed in the sedimentation experiments were measured with capillary viscometers placed in a water bath at 20 or 25 ± 0.1 °C. In the case of deoxycholate this resulted in corrections smaller than 5% of the sedimentation coefficient value. The density of detergent solutions was measured piconometically. Sedimentation values reported are corrected to water at 20 °C.

Diffusion measurements were made employing a capillary synthetic boundary cell in an AnJ rotor at 4800 rpm, and 25 °C, with the interference optics. The protein samples had been rigorously equilibrated with the (detergent-containing) solvent as in the binding experiments. The boundary width at different times was measured from the radial positions of $^1/_4$ and $^3/_4$ of the total fringe displacement (Chervenka, 1969). Viscosity corrections were as in the sedimentation measurements.

Sedimentation equilibrium experiments were made on samples loaded in a cell with a six-channel centerpiece in an AnD rotor at 20 000 rpm and 25 °C. The high-speed procedure (Yphantis, 1964) was employed. Photographs were taken at 1-h intervals after 20 h of centrifugation, and fringe displacement was read at 100- or 200-μm radial intervals, beginning at 50-μm vertical displacement above the meniscus displacement. The partial specific volume of tubulin is 0.735 mL/g (Na & Timasheff, 1981), and the partial specific volume of deoxycholate is 0.778 mL/g (Tanford et al., 1974). The solution can be treated in terms of multicomponent thermodynamics or simply considered to be made of two components, the solvent and the protein–detergent complex whose partial specific volume $\bar{\nu}_c$ is calculated as $\bar{\nu}_c = (\bar{\nu}_p + \delta\bar{\nu}_d)/(1 + \delta)$, were $\bar{\nu}_p$ is the partial specific volume of the protein and $\bar{\nu}_d$ of the bound detergent (assumed to be the same as in detergent micelles) and δ is the grams of detergent bound per gram of protein (Steele et al., 1978). The data are presented in this way here for purely practical reasons. That is, due to the proximity of the protein and detergent partial specific volumes, a relatively large uncertainty in δ (such as ±0.1) results in small differences in $\bar{\nu}_c$ (±0.005 mL/g) and therefore does not contribute large errors to the calculation of the molecular weight of the complex.

Results

Binding of Octyl Glucoside and Deoxycholate to Tubulin. Fluorescence provided the first, and simple, procedure for monitoring the binding of detergents to tubulin. Figure 1 shows the intrinsic fluorescence spectra of tubulin (line a) and the effects of 20 mM octyl glucoside (line b) and 4 mM deoxycholate (line c). A quenching of fluorescence is observed without significant changes in the shape of spectra or the position of the emission maximum at 323 nm. The strong anionic and usually denaturing detergent sodium dodecyl sulfate (6.9 mM, dashed line d) produced clearly different effects, consisting of a more marked reduction in the fluorescence, the appearance of a separate emission peak of tyrosine at 302 nm, and a shift of tryptophan emission to approximately 345 nm. This pointed to a major disruption of the protein structure that can be interpreted as unfolding and exposure of the aromatic residues to the solvent (Chen et al., 1969). The fluorescence quenching by deoxycholate was practically instantaneous, while quenching by octyl glucoside took several minutes. The time course of fluorescence change was pseudo first order as shown in the inset in Figure 1. The rate constant was dependent on the octyl glucoside concentration. At 20 mM octyl glucoside and 25 °C its value was 0.16 min^{-1}.

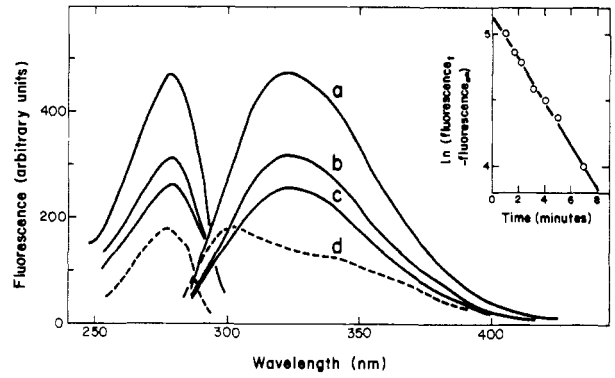

FIGURE 1: Effects of detergents on tubulin fluorescence. Corrected excitation spectra (left) and emission spectra (right) of 53 μg/mL tubulin in 10 mM sodium phosphate–10 μM GTP, pH 7.0, 25 °C, were recorded (Materials and Methods) 20 min after mixing. (a) Tubulin without detergents, (b) with 25 mM octyl gluucoside, (c) with 12 mM deoxycholate, and (d) with 6.9 mM dodecyl sulfate. The inset is a first-order plot of the fluorescence decay time course obtained with 20 mM octyl glucoside.

The extent of fluorescence quenching at equilibrium as a function of ligand concentration is shown in Figure 2 (dark points), where the arrows indicate the critical micelle concentration of the detergents determined under the same experimental conditions. A logarithmic scale of emission intensity is employed in order to linearize the contribution of the inner filter effect (Mertens & Kägi, 1979; Materials and Methods). A sharp sigmoidal transition that is essentially complete when the critical concentration is reached is observed with both detergents. No quenching was observed in the presence of 3.4 M glycerol at 3–4-fold the critical micelle concentration of these detergents, as shown by the open circles in Figure 2. The quenching by dodecyl sulfate was not affected by glycerol. Since glycerol did not significantly change the critical concentration of the detergents (Materials and Methods), this suggested that the inclusion of this cosolvent in the buffer could inhibit the binding of the mild detergents to the protein.

Binding was quantified by application of the Hummel and Dreyer equilibrium gel chromatography technique (Materials and Methods). Representative experiments are shown in Figure 3, profiles a–d. Profile a was obtained by chromatography of tubulin in a column equilibrated with 10 mM [^{14}C]octyl glucoside. A peak of detergent concentration is

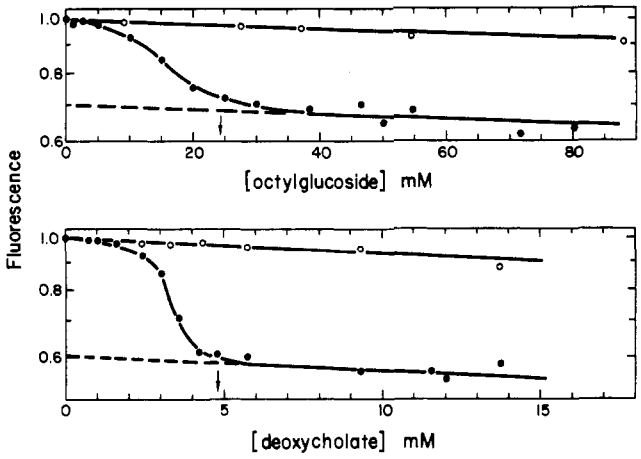

FIGURE 2: Fluorometric titrations of the binding of octyl glucoside and deoxycholate to 106 μg/mL tubulin at 25 °C. Filled circles are data obtained in 10 mM sodium phosphate–10 μM GTP, pH 7.0, while open circles correspond to the same buffer containing 3.4 M glycerol. Emission intensities at 323 nm (excitation at 278 nm) were allowed to reach equilibrium (typically less than 30 min) and divided by the value of controls without detergent. Arrows indicate the critical micelle concentration of each detergent.

observed that was coincident with the elution position of the protein (not shown) and well separated from a later trough. Larger concentrations of octyl glucoside, such as 41.6 mM shown in profile b, gave overlapping maxima and minima in the protein elution zone and did not allow the binding to be measured. Profile c was obtained by chromatography of 44 nmol of tubulin in a column equilibrated with 6 mM deoxycholate and indicated 94 ± 5 mol of detergent bound per mole of tubulin. Since the previous fluorescence measurements suggested an inhibition of detergent binding by glycerol, an experiment identical with profile c, except for the incorporation of 3.4 M glycerol, was performed. It is depicted by profile d and indicated a reduction in the binding to 14 ± 2 molecules of deoxycholate in the presence of the cosolvent. Profiles e and f of Figure 3 are dissociation experiments in which the protein was first expossed to saturating concentrations of detergent and then chromatographed in columns without it. Less than 0.2 molecule of octyl glycoside remained bound to the protein (profile e), but 4.3 ± 0.6 molecules of deoxycholate were still bound (profile f). We were able to reduce the residual deoxycholate by slowing the chromatography, and it decreased to 1.3 molecules when the separation was performed in columns equilibrated with 20 mM octyl glycoside, but it could not be eliminated completely by fast or simple manipulations.

The isotherms binding of octyl glucoside and deoxycholate to tubulin are shown in parts A and B of Figure 4, respectively. The filled circles are column binding measurements, the open circles are fluorescence measurements, and the lines are drawn solely to show the trend of the data. The sharp binding of a large number of detergent molecules in a narrow zone of free ligand concentration is observed in both cases. High-affinity binding at ligand concentrations below 1 mM is probably less than one (deoxycholate) or two (octyl glucoside) detergent molecules. The binding of deoxycholate is essentially saturated at the critical micelle concentration of the detergent. Binding monitored by fluorescence quenching (Figure 2) does not measure the number of detergent molecules bound, ν, but was assumed in a first approximation to measure the fractional saturation of detergent binding sites, $\theta = \nu/n$ (see Materials and Methods). Therefore ν was plotted vs. the fractional fluorescence change $\theta(f)$. An apparently linear relationship was found (correlation coefficient = 0.98) that indicated a number of binding sites at $\theta(f) = 1$ of $n = 95 \pm 8$ (0.39 ± 0.03 g of detergent/g of protein). This number of sites was then employed to scale the right ordinate of Figure 4B. It can be observed that the fluorescence measurements agree within experimental error with the column binding measurements. The same procedure was employed with octyl glucoside (Figure 4A), although in this case column binding measurements could

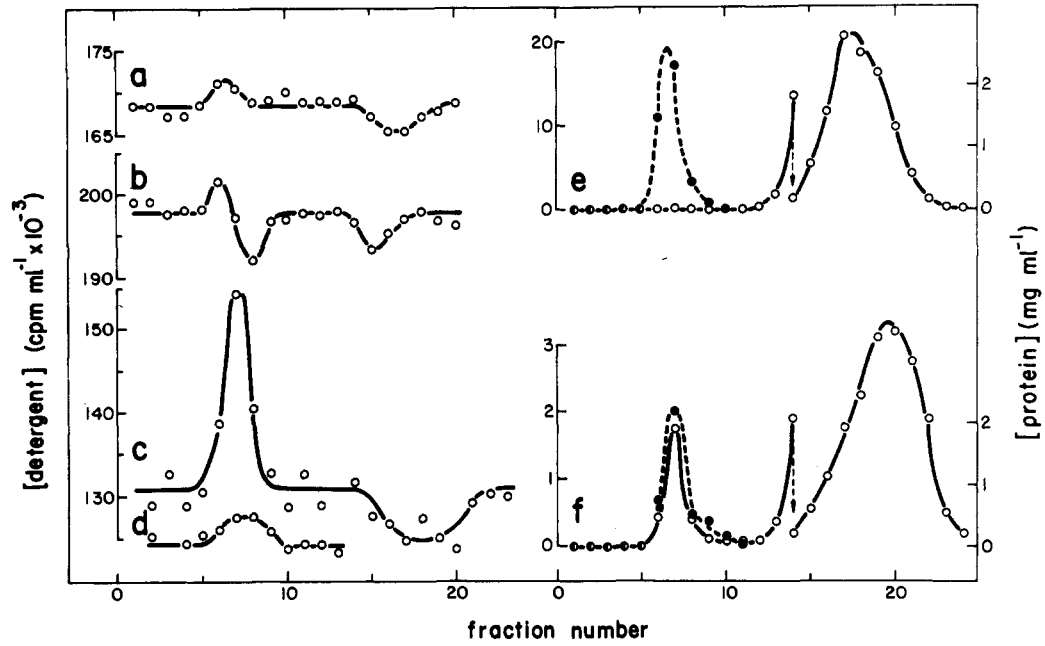

FIGURE 3: Typical results of column binding measurements employing radiolabeled detergents. Equilibrium binding profiles are shown on the left (a–d). Chromatography was in 25 × 0.9 cm Sephadex G-50 columns equilibrated with PG buffer pH 7.0 at 25 °C and flow rates varied between 20 and 40 mL/h. Fractions were 1 mL, and tubulin eluted in fractions 6–8. (a) Chromatography of 42 nmol of tubulin in a column equilibrated with 10 mM octyl glucoside; (b) 42 nmol of tubulin and 41.6 mM octyl glucoside; (c) 44 nmol of tubulin and 6 mM deoxycholate; (d) same as (c) but in buffer containing 3.4 M glycerol. (e) A sample containing 10.9 mg/mL tubulin and 56.5 mM octyl glucoside was held for 10 min at 25 °C and applied to a column without detergent; filled circles are the protein elution profile (right ordinate) and open circles the detergent profile, in which the dashed arrow in fraction 14 indicates a 10-fold scale reduction. (f) A sample containing 10.9 mg/mL tubulin and 21.6 mM deoxycholate chromatographed in a column without ligand.

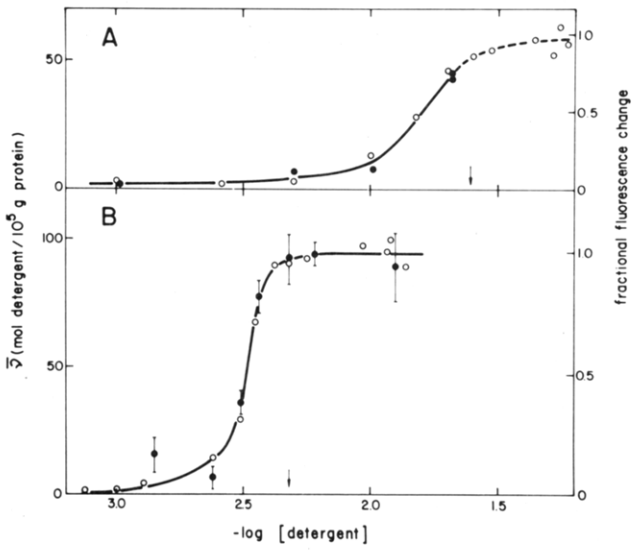

FIGURE 4: Isotherms of binding of octyl glucoside (A) and deoxycholate (B) to tubulin at 25 °C. Filled circles are column binding measurements and open circles fluorescence measurements (right ordinate; see text and Materials and Methods). Arrows indicate the critical micelle concentration of each detergent.

not be made correctly much above 20 mM ligand for the reasons explained above (and in Figure 3b). Therefore, binding saturation could only be estimated by the fluorescence quenching procedure to take place at approximately 60 molecules of octyl glucoside per tubulin (0.18 g of detergent/g of protein). This is a minimal estimation subject to experimental uncertainty and to the fact that the proportionality of binding to fluorescence quenching does not necessarily hold throughout the binding isotherm. Actually, since the detergent induces tubulin self-association (shown below), it is perfectly possible that further octyl glucoside binding linked to the protein aggregation would not give any further fluorescence change. Therefore, the actual number of detergent molecules bound at saturation may be considerably higher than 60. Due to the linkage of binding to protein self-association (Wyman, 1964), high protein concentrations could be expected to shift the apparent binding isotherm of Figure 4A to the left. However, no marked effect of protein concentration in the range employed in these binding studies (0.05–2 mg/mL) was observed. On the other hand, protein self-association was only incipient below the critical micelle concentration of the detergent (Figure 5A). Therefore, it may be concluded that the measurements reported here probably represent the binding of octyl glucoside to nonassociated tubulin. Scatchard (1949) representations of the binding data in Figure 4A,B were strongly convex plots, indicating cooperative binding. Semiempirical analysis of cooperativity by means of Hill plots (see Materials and Methods) gave linear representations of between 10% and 90% saturation approximately, in which a good agreement between fluorescence and column binding measurements was obtained with the sole assumption of $n = 60$ for octyl glucoside. The slopes (Hill coefficients) for the binding of octyl glucoside and deoxycholate were 3.4 and 8.5, respectively.

Tubulin Self-Association Induced by Octyl Glucoside. The large cooperative binding of the mild detergents to tubulin could be the result of binding without changes in protein association, or it could be linked to protein self-association or, on the contrary, it could cause dissociation of the heterodimer with possible denaturation of the α and β chains. To distinguish between these possibilities, a sedimentation velocity study was performed with both detergents.

Figure 5A shows the sedimentation profiles of otherwise rigorously identical 8.24 mg/mL tubulin samples containing 22.6 mM octyl glucoside (total concentration; upper schlieren profile of each frame) or without detergent (lower profiles). These conditions were employed to approximately meet, saving possible protein concentration effects, the middle part of the octyl glucoside binding isotherm. This detergent concentration did not itself give rise to a significant sedimenting boundary but to a small and uniform slope that can be seen to coincide in the schlieren patterns of the sample and reference channels. It can be observed that the detergent induced the formation of a small, fast-sedimenting protein boundary (concentration uncorrected sedimentation coefficient, 11.6 S). The majority of the protein sedimented comparably to the control without detergent (5.7 and 5.4 S, respectively), that under these conditions is the tubulin heterodimer (Lee et al., 1973; Frigon &

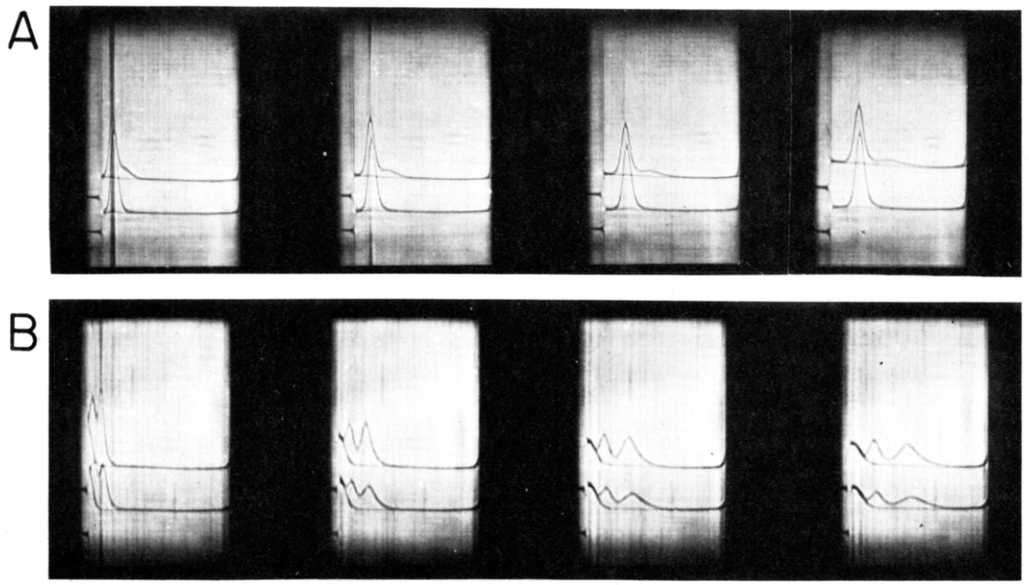

FIGURE 5: Effects of octyl glucoside on the sedimentation velocity of tubulin. (A) 8.24 mg/mL tubulin with 22.6 mM octyl glucoside (upper profiles) and without detergent (lower profiles). Sedimentation is from left to right at 17, 25, 33 (bar angle 70°) and 41 min (bar angle 65°) at a speed of 56 000 rpm and 20 °C. (B) 7.8 mg/mL tubulin (upper profiles) and 4.8 mg/mL tubulin, both with 50 mM octyl glucoside at 8, 16, 24, and 32 min (angle 65°) of sedimentation at 56 000 rpm and 25 °C.

Timasheff, 1975). At this detergent concentration the protein self-association is only incipient, although a considerable number of octyl glucoside molecules are already bound to tubulin (from the total ligand and protein concentrations and the isotherm in Figure 4A, these could be estimated to be 39, with the exception of any possible protein concentration effects). To obtain a qualitative characterization of tubulin self-association induced by octyl glucoside, similar sedimentation velocity runs were made at detergent concentrations saturating the fluorescence titration. Clearly bimodal patterns were observed. The area under the two peaks was found to be approximately 1.3 times larger than the area under the single peak obtained with identical samples without detergent that were run simultaneously (not shown). Figure 5B shows the sedimentation profile of 4.8 (lower profile of each frame) and 7.8 mg/mL (upper profile) tubulin with 50 mM octyl glucoside. At this concentration the detergent gave a noticeable peak sedimenting more slowly than the protein. Since most of the detergent is not bound to the protein, this peak, probably corresponding to detergent micelles, was nearly coincident in the sample (protein + detergent) and reference (detergent) channels, and the semiquantitative observation of the protein sedimentation pattern was possible. The bimodal pattern was evident from the beginning of the run, and the boundaries sedimented at 5.4 and 12 S (concentration uncorrected). The trough between the peaks was not observed to reach the base line at long sedimentation times. Moreover, increasing the protein concentration from 4.8 to 7.8 mg/mL increased the ratio of the area of the fast peak to the area of the slow peak from 1.4 to 1.8 approximately. These characteristics indicated a fast-equilibrating self-association reaction of the protein, in accordance with the diagnostic criteria discussed by Prakash and Timasheff (1983), that might be of the Gilbert type (Gilbert & Gilbert, 1973). The uncorrected sedimentation coefficient of the slow-moving peak suggests that the associating species may be the tubulin heterodimer. The sedimentation rate of the fast-moving peak indicates that the association products are probably larger than two tubulin heterodimers.

Increased Frictional Coefficient of the Tubulin–Deoxycholate Complex. In contrast to octyl glucoside, binding of deoxycholate to tubulin produced a decrease in its sedimentation velocity. Figure 6A shows the sedimentation profile of 5.1 mg/mL tubulin with 20 mM deoxycholate (upper schlieren profile) compared to the same tubulin concentration without detergent (lower profile). This high detergent concentration produced by itself a slowly sedimenting boundary giving a skew appearance to the tubulin peak. Since the base-line region of the sample profile was coincident with the reference profile, use of the latter to make a base-line correction gave a symmetrical peak, clearly sedimenting more slowly than in the absence of detergent. This was confirmed in the experiment of Figure 6B which shows the ultraviolet absorption profiles of 1.45 mg/mL tubulin sedimenting with and without deoxycholate. The peak in the presence of detergent is clearly symmetrical and has the same area, although it is slightly wider than in the absence of deoxycholate. In three experiments employing the schlieren optics with saturating deoxycholate concentrations, the area under the protein peak in the presence of detergent was 1.50 ± 0.08 times that of exactly the same protein concentration run simultaneously without detergent. Such an increase in the effective refractive index of the protein provided an uncalibrated measurement of the massive detergent binding, just as in the case of octyl glucoside. Figure 6C (upper profile) shows the sedimentation profile of 6.3 mg/mL

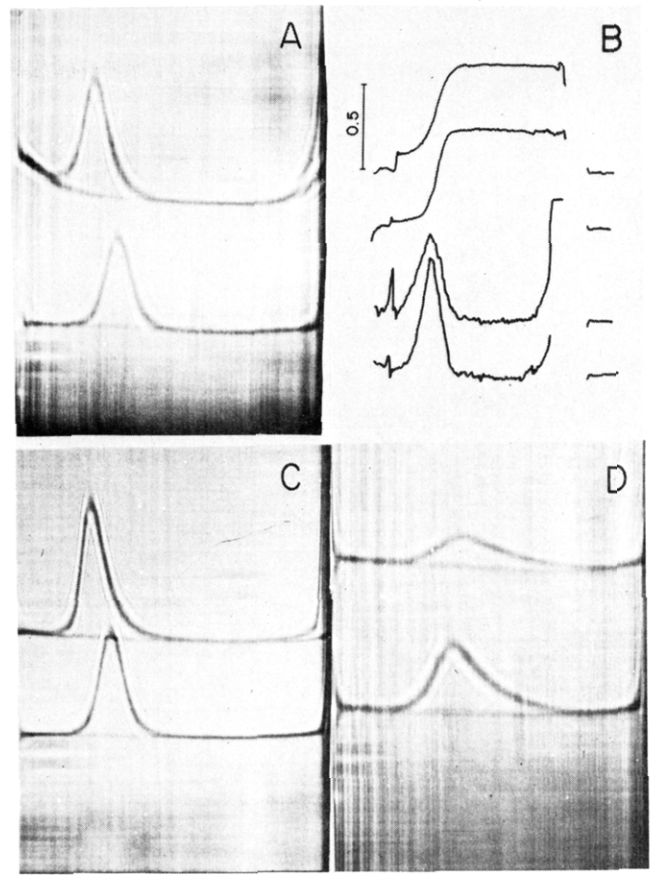

FIGURE 6: Effects of deoxycholate on the sedimentation velocity of tubulin. (A) 5.1 mg/mL tubulin with 20 mM deoxycholate (upper profile) and without it (lower profile); 56 min at 56 000 rpm, 20 °C (bar angle 60°). (B) 1.45 mg/mL tubulin with 20 mM deoxycholate (upper profile and upper derivate profile, 49 min) and without detergent (lower profile and lower derivative profile, 45 min); 56 000 rpm at 20 °C, wavelength 294 nm. (C) 6.3 mg/mL tubulin with 12 mM deoxycholate (upper profile) and without it (lower profile); 56 min at 56 000 rpm, 20 °C (bar angle 65°). (D) 2.8 (upper profile) and 4.8 mg/mL (lower profile) tubulin equilibrated with 3.8 mM free deoxycholate; 65 min at 56 000 rpm, 25 °C (bar angle 55°).

tubulin with 12 mM total deoxycholate, giving an estimated free detergent concentration of approximately 6 mM (essentially saturating the isotherm of Figure 4B), that was matched by 6 mM deoxycholate in the reference channel. Here the interference by unbound detergent in the schlieren pattern was minimal. Tubulin sedimented as a slightly asymmetrical peak, clearly more slowly than tubulin without detergent (lower profile of Figure 6C). A shoulder sedimenting at a rate roughly comparable to tubulin was clearly observed at longer times (not shown). In order to obtain conditions rigorously corresponding to the middle region of the deoxycholate binding isotherm (Figure 4B), tubulin was equilibrated with 3.8 mM deoxycholate by column chromatography at 25 °C, in a similar way to the binding experiments. Figure 6D shows the sedimentation profiles of 2.8 (upper) and 4.8 mg/mL (lower) tubulin equilibrated with the detergent. An asymmetrical, rapidly spreading boundary was observed in both cases, possibly indicating the presence of fast- and slow-moving components that were not resolved.

The sedimentation coefficient of the tubulin–deoxycholate complex was determined on protein solutions containing a detergent excess. Figure 7 shows the concentration dependence of the viscosity- and density-corrected sedimentation coefficient (see Materials and Methods) of tubulin–deoxycholate (filled circles) compared to that of tubulin (open circles). The

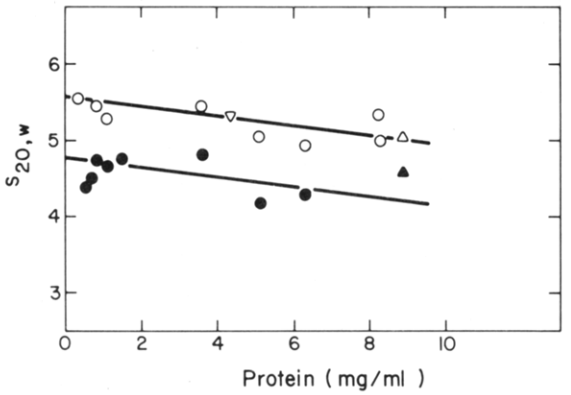

FIGURE 7: Concentration dependence of the sedimentation coefficient of tubulin with excess deoxycholate (filled symbols) compared to tubulin (open symbols). Measurements were made at 20 or 25 °C and corrected for viscosity and density (Materials and Methods). Results of experiments with three different tubulin preparations are plotted. Deoxycholate concentrations varied between 12, 16, and 20 mM, keeping the free detergent concentration ⩾6 mM to ensure binding saturation, according to the data in Figure 4B. Triangles represent measurements on protein hold for 24 h at 25 °C (Figure 9A) and the inverted triangle is a measurement on tubulin from which octyl glucoside had been released (Figure 9B, upper profile). Measurements below 2 mg/mL were made employing the photoelectric scanner.

concentration-corrected values were respectively 4.8 ± 0.3 and 5.6 ± 0.2 S. The latter value is in agreement with previous measurements [5.8 ± 0.2 S (Frigon & Timasheff, 1975)].

Since the binding of deoxycholate confers more mass to tubulin (0.39 ± 0.03 g/g), the observed reduction in the sedimentation coefficient could only be met by two possibilities: (i) dissociation of the tubulin heterodimer to the detergent-bound subunits (which would give a change of molecular weight from about 100 000 to an average of 69 500 in the tubulin–detergent complexes) or (ii) a considerable increase in the frictional coefficient without dissociation. To distinguish between these two opposite possibilities, the sedimentation equilibrium of tubulin in the presence of deoxycholate was examined. At first these measurements were expected to prove difficult, since sedimentation of the detergent with interference in the Rayleigh pattern had to be avoided. On the other hand, tubulin is known to dissociate at low concentrations (Detrich et al., 1982). These effects give rise to heterogeneous sedimentation equilibrium, requiring a detailed analysis in terms of association–dissociation models (Detrich et al., 1982). They may produce clearly nonlinear plots of ln (concentration) vs. (radial distance)^2 such as the example shown by the open circles of Figure 8. Addition of excess deoxycholate was found to remove nonlinearity. Adequate free detergent concentrations essentially saturating binding at 25 °C (6.03 ● 0.07 mM deoxycholate) did not give significant fringe displacement in the absence of protein (less than 10 μm at the bottom of the cell in interference pattern b of Figure 8). The equilibrium distribution of the tubulin–deoxycholate complex, shown by interference pattern a and solid circles of Figure 8, indicated apparent homogeneity. The calculation of the partial specific volume of the complex was only slightly influenced by the exact number of detergent molecules bound, due to the proximity of the partial specific volumes of tubulin and deoxycholate. Therefore, possible errors in the binding measurements caused changes in the estimation of molecular weight that are within the experimental error reported (see Material and Methods). Determinations at initial protein concentrations 0.5 and 1 mg/mL gave a molecular weight of 144 000 ± 5000 for the protein–detergent complex. This number is clearly not compatible with dissociation to detergent-bound α and β subunits

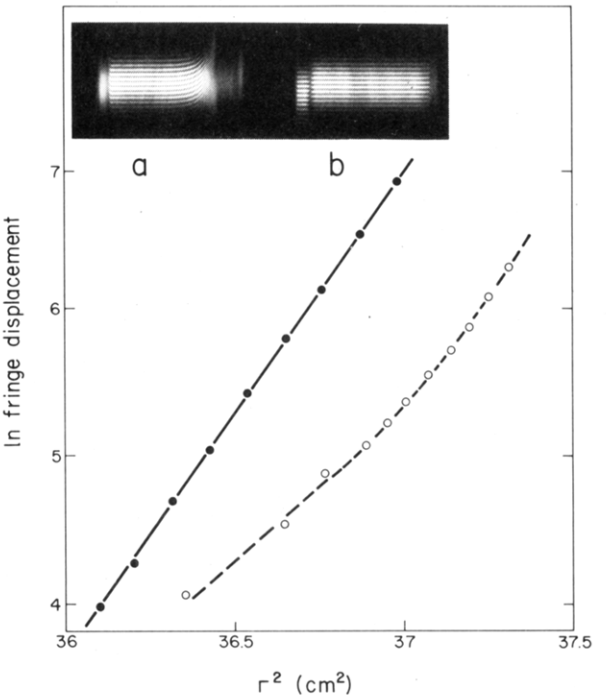

FIGURE 8: Sedimentation equilibrium of 0.5 mg/mL tubulin at 20 000 rpm, 25 °C, with 6.03 ± 0.07 mM free deoxycholate (interference profile a and filled circles) and without detergent (open circles). Interference profile b was obtained with detergent without protein. The points plotted are averages of two essentially coincident successive plates at 1-h intervals.

but affords a measurement of detergent binding to the heterodimer (0.44 ± 0.05 g of detergent/g of protein) which is in good agreement with the chromatographic binding measurements (0.39 ± 0.03 g of detergent/g of protein). Larger detergent concentrations did not give a rigorously flat interference profile in the absence of protein. In the presence of protein the upper two-thirds of the profile were flat, and the molecular weights obtained for the tubulin–detergent complex, obviously not accurate, were close to 150 000. Since sedimentation equilibrium measurements were made after 20 h of tubulin centrifugation, and because of the possible pitfalls mentioned earlier, it was desirable to confirm the molecular weight obtained by an independent measurement involving shorter experimental times. For this purpose, the diffusion of tubulin rigorously equilibrated both in buffer and in buffer containing deoxycholate was approximately measured in the ultracentrifuge at 25 °C (Materials and Methods). The diffusion coefficient of 3 mg/mL tubulin without detergent was $D_{20,w} = (5.3 \pm 0.5) \times 10^{-7}$ cm^2 s^{-1} which, assuming a concentration dependence similar to the sedimentation velocity (Figure 7; Frigon & Timasheff, 1975), is within an experimental error of $D^0_{20,w}$. This value is in excellent agreement with the diffusion coefficient calculated for tubulin, $(5.2 \pm 0.2) \times 10^{-7}$ cm^2 s^{-1}. Diffusion measurements performed on 1.1 and 2.3 mg/mL tubulin equilibrated with 6 mM free deoxycholate did not show any significant trend with concentration. The average value obtained was $D^0_{20,w} = (3.8 \pm 0.2) \times 10^{-7}$ cm^2 s^{-1}, in agreement with the diffusion coefficient calculated for the detergent-bound heterodimer, $D^0_{20,w} = (3.2 \pm 0.4) \times 10^{-7}$ cm^2 s^{-1}, but not compatible with the value calculated for the monomers, $D^0_{20,w} = (6.4 \pm 0.2) \times 10^{-7}$ cm^2 s^{-1}. The hydrodynamic characteristics of tubulin-detergent complexes are summarized in Table I for the purpose of comparison.

Effects of Deoxycholate on the Spontaneous Aggregation of Tubulin. Reversibility of the Octyl Glucoside Induced Self-Association. Tubulin is known to undergo an spontaneous

Table I: Hydrodynamic Characteristics of the Tubulin–Detergent Complexes

detergent	maximal binding (g of detergent/g of protein)	$s^0_{20,w}$ (S)	M_r	f/f_{min}[c]	$D_{20,w} \times 10^7$ (cm^2/s)
none		5.6 ± 0.2	100 000[a]	1.35	5.3 ± 0.5 (5.2 ± 0.2)
deoxycholate	0.39 ± 0.03	4.8 ± 0.3	144 000 ± 5000[b] (139 000 ± 4000 for dimer or 68 000 ± 2000 for monomer)	1.86	3.8 ± 0.2 (3.2 ± 0.4 for dimer or 6.4 ± 0.9 for monomer)
octyl glucoside	0.18	bimodal (approximately 5 and 12 S)			

[a] From the amino acid sequence of porcine tubulin (Krauhs et al., 1981). [b] Measured by sedimentation equilibrium (values in parentheses were calculated from the binding data). [c] Calculated from $s^0_{20,w}$ and the molecular weights.

relaxation of its secondary structure (Andreu & Timasheff, 1982) and a slow aggregation with the production of fast-sedimenting components that may be due in part to the exposure of hydrophobic regions (Prakash & Timasheff, 1982). It was of interest to know the effect of detergent on this process and also to verify whether the sedimentation velocity of the tubulin–deoxycholate complex was stable with time under conditions similar to those employed in the sedimentation equilibrium. Therefore, the effects of a 24-h preincubation at 25 °C on the sedimentation pattern of tubulin with 14.5 mM total deoxycholate (estimated free detergent, 6.0 ± 0.9 mM) were examined, and the results are shown in Figure 9A (upper profile of each frame). A single, slightly asymmetrical peak was observed with $s_{20,w}$ = 4.7 S (9 mg/mL). This is in aggreement with previous observations (Figure 6C) and within the experimental error of the sedimentation coefficient of the tubulin–deoxycholate complex without aging (Figure 7, filled triangle). In contrast to this, and in qualitative agreement with the results reported by Prakash and Timasheff (1982), tubulin preincubated for 24 h at 25 °C without detergent was partially converted to faster sedimenting components (27 S) as shown by the lower profile in each frame of Figure 9A.

In a different experiment, Figure 9B shows the effects of saturating tubulin with detergents and then removing them by gel chromatography. In the case of octyl glucoside (upper profile of each frame) a symmetrical peak with $s_{20,w}$ = 5.3 S (4 mg/mL) plus a minor (approximately 10%) fast-sedimenting component were observed. This indicated that the majority of the protein had regained the sedimentation velocity of native tubulin (inverted triangle in Figure 7) upon the removal of essentially all detergent (Figure 3e). Saturation of tubulin with deoxycholate and partial removal of detergent, leaving approximately four molecules bound to tubulin (Figure 3f), did not result in the species sedimenting like native tubulin but in a rapidly spreading boundary that sedimented at 9.5 S, indicating aggregation of the protein by the detergent-removal procedure.

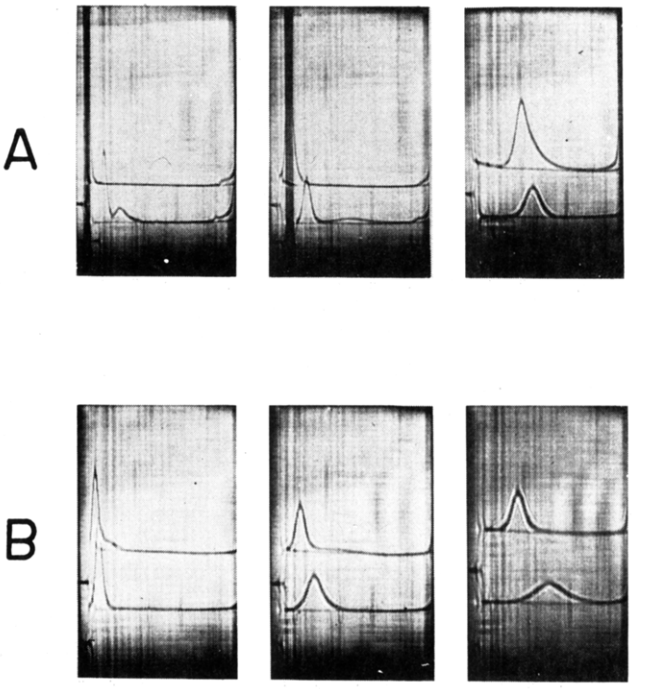

FIGURE 9: (A) Sedimentation velocity patterns of 9 mg/mL tubulin hold for 24 h at 25 °C with 14.5 mM deoxycholate (upper profiles) and without detergent (lower profiles). Pictures were taken at 4, 12 (bar angle 70°), and 56 min (angle 65°) of centrifugation at 56 000 rpm and 25 °C. (B) Upper profiles: 4.3 mg/mL tubulin previously saturated with octyl glucoside and from which detergent has been released by chromatography. Lower profiles: 4.2 mg/mL tubulin previously saturated and dissociated from deoxycholate. Pictures were taken at 8, 24 (angle 60°), and 48 (55°) min of centrifugation at 56 000 rpm and 25 °C.

Discussion

Hydrophobic Binding of Mild Detergents to Tubulin. Effects of Glycerol. This paper demonstrates the binding of large amounts of octyl glucoside and deoxycholate to purified cytoplasmic calf brain tubulin. Binding is cooperative and fully reversible (octyl glucoside) or 95% reversible (deoxycholate).

Binding was observed to proceed just below the critical micelle concentration of each detergent. Following the reasoning exemplified by Reynolds (1979), this suggests that the cooperative binding of detergent monomers to the protein is slightly preferred thermodynamically over the binding of detergent molecules among themselves to form micelles. The binding of octyl glucoside to tubulin could be due to a nonspecific interaction of the glucose sugar moieties of the detergent with the protein. However, this seems unlikely, since the high binding cooperativity would be difficult to explain. More important, tubulin is known to be stabilized and preferentially to exclude sucrose from its vicinity (Lee et al., 1975). This general thermodynamic effect has been extended to other proteins (Lee & Timasheff, 1981) and other sugars such as lactose and glucose (Arakawa & Timasheff, 1982). On the other hand, the binding of deoxycholate to tubulin could be the results of exclusively ionic interactions. However, both this detergent and tubulin are anionic. Actually, due to the proximity to the pK of the detergent, it is not known whether the species that binds to tubulin is mainly the deoxycholate anion or the un-ionized deoxycholic acid. The massive, cooperative and nonspecific binding of model amphiphiles to a protein without a drastic conformational change is most simply interpreted as due to the presence of accessible hydrophobic regions in a folded structure of the protein; this constitutes a criterion for the identification of such protein as lipophilic (Helenius & Simmons, 1975; Tanford & Reynolds, 1976;

Helenius et al., 1979; Tanford, 1980). The fluorescence changes induced by the mild detergents consisted of quenching, without other noticeable changes that might indicate tubulin denaturation. Quenching does not necessarily imply a conformational change of tubulin, but simply binding near tryptophan residues. This is in agreement with the perturbation by detergents of the protein light absorption in the near-ultraviolet region, reported previously (Andreu, 1982).[2] On the other hand, the strong anionic detergent sodium dodecyl sulfate caused typically denaturational fluorescence changes. Therefore, the binding results obtained with deoxycholate and octyl glucoside strongly suggest the existence of large regions of tubulin to which the apolar moieties of these mild detergents bind hydrophobically.

Binding of the mild detergents to tubulin was markedly inhibited in the presence of 3.4 M glycerol. This cosolvent was found to have no significant effects on the critical micelle concentrations of the detergents, which are measurements of their ability to interact hydrophobically and ultimately, functions of the standard free energy change of transfer of the detergent monomer from the solution into the micelles (Tanford, 1980). Therefore, it may be suggested that glycerol exerts its effect by interacting with the protein. This cosolvent is known to stabilize tubulin and to enhance its polymerization into microtubules. This is quite probably due to a nonspecific thermodynamic effect that consists in the preferential exclusion of glycerol from the protein domain, which tends to favor the states in which the protein–solvent contact is minimized, such as the folded or polymerized state (Lee & Timasheff, 1977). It was actually shown by Na and Timasheff (1981) that the apparent partial specific volume of tubulin measured at constant chemical potential of dialyzable components increased with increasing glycerol concentrations, while the protein partial specific volume at constant molality of glycerol remained practically constant. Application of multicomponent solution thermodynamics made it possible to calculate the preferential interaction parameter of glycerol with tubulin, which is indeed negative (Na & Timasheff, 1981). In the present case, what is the significance of the fact that glycerol hampers the association of mild detergents to tubulin? To achieve this effect, the introduction of the cosolvent must destabilize the tubulin–detergent complex. This unfavorable effect need not be large, but in fact, raising a fraction of kilocalorie per mole of amphiphile the chemical potential of the protein–detergent complex might be enough to make its formation less favorable than the micelle formation reaction, so that massive detergent binding to the protein would not be observed. In other words, the hypothetical transfer reaction of the tubulin–detergent complex from water to a water–cosolvent solution must be favored even less than the transfer of tubulin, for which Na and Timasheff (1981) estimated a positive standard free energy change of 73 kcal/mol. Following their line of reasoning, the simplest interpretation of the effect of glycerol on the interaction of tubulin with the detergents is that there should be an increase in the area of solutes exposed to solvent upon formation of the tubulin–detergent complex. Since glycerol does not apparently affect the self-association of detergent, it might be predicted on these and the above-mentioned exclusively thermodynamic considerations that the binding of the amphiphiles to tubulin should induce dissociation or a limited unfolding of this protein. This would be prevented by glycerol only with the mild detergents but not with the strong denaturing detergent dodecyl sulfate.

Hydrodynamics of the Tubulin–Detergent Complexes. The main effects of deoxycholate binding on the tubulin sedimentation velocity consisted in a slowing from 5.6 ± 0.2 to 4.8 ± 0.3 S. This was not due to dissociation to α and β subunits but to a marked increase in the frictional coefficient of the tubulin heterodimer–detergent complex (Results and Table I). The frictional coefficient ratio determined for the tubulin heterodimer, $f/f_{min} = 1.35$, is in agreement with a value previously calculated on a different set of hydrodynamic measurements ($f/f_{min} = 1.41$, and, accounting for hydration, $f/f_0 = 1.24$; Frigon & Timasheff, 1975). It is also consistent with the radius of gyration of tubulin determined by X-ray scattering (3.1 nm, affording $f/f_{min} = 1.30$; Bordas et al., 1983). The frictional ratio of the tubulin–deoxycholate complex, 1.86, clearly indicates a departure from the class of the compact globular proteins (Tanford, 1961, pp 356–361). It is significantly larger than the frictional ratios reported for the deoxycholate complexes of some membrane proteins such as the hydrophobic fragment of cytochrome b_5 (1.2; Visser et al., 1975) and Ca^{2+} from sarcoplasmic reticulum (1.4–1.6; Le Maire et al., 1976). The increased frictional coefficient of tubulin–deoxycholate could be the result of increased hydration or expansion (Tanford, 1961). Hydration alone would require abnormally high values (4 g of water/g of tubulin–deoxycholate). Assuming moderate hydration, the results suggest either an increase in asymmetry or a certain unfolding of the protein. The axial ratio for compact ellipsoids of revolution would be 14–20, which favors the latter possibility. On the basis of the present data, no distinction can be made between the two possibilities. The second one would be consistent with the prediction on the basis of purely thermodynamic grounds that the binding of deoxycholate might cause an increase in the surface area of tubulin exposed to solvent. This has been clearly supported by the moderate circular dichroism changes and the increased susceptibility to proteolysis that are similarly observed when deoxycholate or octyl glucoside binds to tubulin (Andreu 1986; Andreu et al., 1986). The hydrodynamic expansion may equally take place when octyl glucoside binds to tubulin. However, the sedimentation velocity profiles are dominated by the major effect of detergent-induced tubulin self-association, which precludes adequate observations on the heterodimer.

The inhibition by deoxycholate of the spontaneous aggregation of tubulin (Prakash & Timasheff, 1982) indicates the possible participation of hydrophobic interactions between partially unfolded protein molecules. While partial removal of deoxycholate from the tubulin–detergent complex resulted in protein aggregation, the essentially complete removal of octyl glucoside reverted the protein self-association induced by this detergent, restoring the normal sedimentation behavior to 90% of the protein. Octyl glucoside is a mild and easily removed detergent that has been successfully employed for the reconstitution (Helenius et al., 1977) and crystallization of membrane proteins (Michel, 1983). This detergent has been recently used for the effective solubilization of the tubulin-containing protein–lipid complex of ciliary membranes (Stephens, 1985a) which is resistant to Triton X-114 (Stephens, 1985b).

Structural Change of Cytoplasmic Tubulin from Water-Soluble to Amphipathic Protein and the Membrane Tubulin

[2] The present results extend and confirm the results obtained in that study with deoxycholate and octyl glucoside. However, difference spectra very similar to those previously obtained with Triton X-100 and tubulin [Figure 3C in Andreu (1982)] can be generated by micellization of this phenolic detergent in the absence of protein. Therefore, the implicit conclusion that Triton binds to tubulin, based exclusively on those light absorption results, is incorrect and is now withdrawn.

Problem. The results discussed above clearly indicate that tubulin, an otherwise globular water-soluble protein, reversibly binds large amounts of amphiphilic compounds, most probably to apolar regions. Hydrophobic binding induces a hydrodynamically detected structural change, suggested to be a loosening of the protein structure. This is clearly different from denaturation by dodecyl sulfate. In the case of octyl glucoside, binding is fully reversible, and it has been shown that amphiphile dissociation results in the recovery of the colchicine binding and microtubule assembly properties of tubulin (Andreu, 1986). It is clear that cytoplasmic tubulin is different from typical intrinsic membrane proteins, which have exposed hydrophobic regions and agggregate in the absence of lipid or detergents. Therefore, tubulin must exist in equilibrium between the water-soluble form and the amphipathic forms induced by binding of mild detergents. In these latter forms, previously nonaccesible apolar areas must become exposed or accesible to the hydrophobic ligands. Such a remarkable property as this water-soluble/amphipathic protein switching indicates that tubulin is a very flexible molecule. It could be a reflection of the ability to form hydrophobic contacts between protomers in microtubule assembly. On the other hand, this property is relevant to membrane tubulin (see introduction): (i) It constitutes an explanation for the apparent ability of cytoplasmic tubulin to be inserted into phospholipid bilayers in vitro. (ii) It provides a rationale for the repeatedly reported existence of membrane proteins closely related to cytoplasmic tubulin. If these proteins were produced by posttranslational modification coupled to membrane insertion of cytoplasmic tubulin, it should be possible to biosynthetically trace the interconversion between the cytoplasmic pool and the hypothetical membrane pool and to identify the type of modification, in a similar way to the recently elucidated cytoplasmic ↔ axoplasmic tubulin system of Chlamydomonas (L'Hernault & Rosembaum, 1985). (iii) It cautions against possible microtubular contamination during the solubilization of membrane tubulin, since both forms bind detergents. (iv) Finally, the experimental conditions established for the reversible interaction of cytoplasmic tubulin with octylglucoside could be tested for the solubilization and purification of membrane tubulin. An obvious question is whether the latter protein would be able to associate into microtubule-related structures upon controlled detergent removal.

The effects of the binding and dissociation of mild detergents on the structure of cytoplasmic tubulin in solution and its ligand and self-assembly properties are the subject of the accompanying paper (Andreu et al., 1986).

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Registry No. Deoxycholate, 83-44-3; octyl glucoside, 29836-26-8.

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Interaction of Tubulin with Octyl Glucoside and Deoxycholate. 2. Protein Conformation, Binding of Colchicine Ligands, and Microtubule Assembly†

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ABSTRACT: The structural change induced by binding of mild detergents to cytoplasmic calf brain tubulin and the effects on the functional properties of this protein have been characterized. Massive binding of octyl glucoside or deoxycholate monomers induces circular dichroism changes indicating a partial α-helix to disordered structure transition of tubulin. The protein also becomes more accessible to controlled proteolysis by trypsin, thermolysin, or V8 protease. This is consistent with the looser protein structure proposed in previous binding and hydrodynamic studies [Andreu, J. M., & Muñoz, J. A. (1986) *Biochemistry* (preceding paper in this issue)]. Micelles of octyl glucoside and deoxycholate bind colchicine and its analogue 2-methoxy-5-(2,3,4-trimethoxyphenyl)-2,4,6-cycloheptatrien-1-one (MTC). This impedes the determination of colchicine binding in the presence of detergents. Both detergents cause a reduction in the number of tubulin equilibrium binding sites for the colchicine site probe MTC. Deoxycholate monomers bind poorly to the tubulin–colchicine complex, but deoxycholate above the critical micelle concentration effectively dissociates the complex. Microtubule assembly in glycerol-containing buffer is inhibited by octyl glucoside, which raises the critical protein concentration. Low concentrations of deoxycholate enhance tubulin polymerization, allowing it to proceed without glycerol. The polymers formed are microtubules, pairwise associated open microtubular sheets, and macrotubes possibly generated by helical folding of the sheets, as indicated by the optical diffraction patterns. Saturation of tubulin with octyl glucoside, followed by full dissociation of the detergent, allowed the recovery of binding to the colchicine site and microtubule assembly, indicating the reversibility of the protein structural change.

Cytoplasmic tubulin from bovine brain, a water-soluble protein, is able to show extensive hydrophobic interactions. Tubulin associates to unilamellar dipalmitoylphosphatidylcholine vesicles (Klausner et al., 1981), where it is apparently inserted into the lipid bilayer (Kumar et al., 1981). It has been further reported that vesicle-bound tubulin exhibits normal colchicine and MAP binding and is able to mediate the nonleaky membrane fusion of the vesicles induced by calcium ions

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