A Synchrotron X-ray Scattering Characterization of Purified Tubulin and of Its Expansion Induced by Mild Detergent Binding†

José Manuel Andreu,*,‡ Jorge Garcia de Ancos,‡ David Starling,§,‖ Julie Lynne Hodgkinson,§ and Joan Bordas‖
Centro de Investigaciones Biológicas, CSIC, Velâzquez 144, 28006 Madrid, Spain, Department of Physics, Liverpool Polytechnic, Byrom Street, Liverpool, U.K., and SERC Daresbury Laboratory, Daresbury, Warrington, Cheshire, U.K.
Received December 16, 1988

ABSTRACT: This report presents a synchrotron radiation X-ray scattering characterization of calf brain tubulin purified by the modified Weisenberg procedure. The results show that under nonassembly conditions (i.e., in 10 mM sodium phosphate and 0.1 mM GTP, pH 7, buffer) these preparations consist of a uniform population of molecules with a radius of gyration of 3.1 ± 0.1 nm, which can be interpreted as arising from the native α–β heterodimer. The uniformity in the population persists even at unusually high concentrations of protein. Binding of colchicine or substitution of GTP by GDP does not induce, within the statistical accuracy and resolution range of our measurements, any significant structural modification in soluble tubulin. In assembly buffer [i.e., 10 mM sodium phosphate, 6 mM magnesium chloride, 1 mM [ethylenebis(oxyethylenenitrilo)]tetraacetic acid, 1 mM GTP, and 3.4 M glycerol, pH 6.5], these preparations readily assemble into microtubules upon increasing the temperature from 4 to 37 °C. Binding of nondenaturing amphiphiles to soluble tubulin provides a simplified model for tubulin–membrane interactions. The X-ray scattering data show that the radius of gyration of tubulin progressively increases upon binding of the mild detergent sodium deoxycholate, reaching a maximum value of 4.3 ± 0.1 nm at detergent saturation. The relative increase in the radius of gyration coincides within experimental error with the previously determined relative increase in the frictional coefficient [Andreu, J. M., & Muñoz, J. A. (1986) *Biochemistry 25*, 5220–5230]. Analysis of these observations suggests that the effect of detergent binding is to induce an isotropic swelling of the protein structure.

Time-resolved X-ray scattering using synchrotron radiation (SR)[1] has provided important insights into the mechanisms of microtubule assembly from pig brain microtubule protein (Mandelkow et al., 1980, 1988; Bordas et al., 1983; Mandelkow & Bordas, 1986; Spann et al., 1987). In these studies the initial state was a mixture of oligomers made of tubulin and MAPs, and before microtubule assembly could proceed, it was necessary for these oligomers to dissociate into smaller species and then be activated for microtubule nucleation and elongation (Spann et al., 1987). While microtubule protein has been extensively studied by X-ray scattering methods, the characterization of phosphocellulose-purified pig brain tubulin and MAP-free microtubules is far less extensive (Bordas et al., 1983; Mandelkow, 1986).

Purified calf brain tubulin, on the other hand, has been thoroughly characterized by conventional biophysical approaches (Lee et al., 1973; Frigon & Timasheff, 1975; Na & Timasheff, 1981, 1982; Lee, 1982; Howard & Timasheff, 1986) and its assembly into microtubules carefully studied (Lee & Timasheff, 1975, 1977; Timasheff, 1981). Nondenaturing detergents bind extensively to purified calf brain tubulin, and this property has been employed to generate a simplified solution model of tubulin–membrane interactions (Andreu, 1982, 1986). From the results of hydrodynamic and conformational measurements it has been proposed that the tubulin α–β heterodimer expands, without dissociating to monomers, when the detergent binds to normally hidden hydrophobic regions of the protein (Andreu & Muñoz, 1986; Andreu et al., 1986).

This report presents a study of purified calf brain tubulin using SR techniques. The aims of this study are (i) to characterize by this technique the tubulin heterodimer in solution, the possible effects of ligands and high protein concentrations, and the ability of this tubulin preparation to assemble into microtubules and (ii) to verify and measure the proposed expansion of the tubulin heterodimer induced by deoxycholate binding (Andreu & Muñoz, 1986).

Materials and Methods

Calf brain tubulin was purified, stored in liquid nitrogen, and prepared for use and its concentration measured spectroscopically as described previously (Weisenberg et al., 1968; Lee et al., 1973; Andreu et al., 1984). Sodium deoxycholate was from Calbiochem (lot no. 310018), GTP and GDP were from Boehringer-Mannheim, colchicine was from Aldrich, Sephadex G-25 and G-50 were from Pharmacia, ultrapure guanidinium chloride was from BDH, and glycerol was from Merck.

The R_G measurements were performed in PG buffer, pH 7.0. Measurements to test the ability of the protein to assemble into microtubules were carried out in assembly buffer. Immediately prior to the measurements all the samples were centrifuged at 4 °C for 20 min at 200000*g*. Tubulin was equilibrated with different concentrations of free deoxycholate by chromatography in Sephadex G-50 columns, which had

† This work was supported by the SERC's Synchrotron Radiation Facility Committee through the SP01 beam time award, Spanish DGICyT Grant PB0220, and travel funds from DGICyT and Departamento Relaciones Internacionales CSIC.
‡ CSIC.
§ Liverpool Polytechnic.
‖ SERC Daresbury Laboratory.

[1] Abbreviations: assembly buffer, 10 mM sodium phosphate, 6 mM magnesium chloride, 1 mM [ethylenebis(oxyethylenenitrilo)]tetraacetic acid, 1 mM GTP, and 3.4 M glycerol, pH 6.5; GDP, guanosine 5′-diphosphate; GTP, guanosine 5′-triphosphate; MAPs, microtubule-associated proteins; R_G, radius of gyration; PG, 10 mM sodium phosphate and 0.1 mM GTP, pH 7.0; SR, synchrotron radiation; SRS, synchrotron radiation source.

0006-2960/89/0428-4036$01.50/0

been equilibrated with the detergent (Andreu & Muñoz, 1986). Binding of deoxycholate to tubulin was monitored by the quenching of the intrinsic fluorescence of the protein. The fractional quenching effect is proportional to the number of detergent molecules bound per tubulin heterodimer (Andreu & Muñoz, 1986), and it is therefore considered here, in reasonable approximation, equal to the fractional saturation of binding.

The X-ray scattering traces were recorded by using a double-focusing X-ray camera (station 2.1) sited at the synchrotron radiation source (SRS) at the Daresbury Laboratory (Daresbury, England). The specifications for this camera are given elsewhere (Towns et al., 1988). The camera is equipped with a data acquisition system along the lines described by Bordas et al. (1980). A quadrant detector system (Lewis et al., 1988) was used for the measurements. For these experiments the camera was set up to cover a range of scattering vectors from ca. 0.02 to 0.13 mm^{-1}. A wavelength of 0.157 nm was used. Calibration of the scattering vectors S [defined as $2(\sin \Theta)/\lambda$] was obtained from the diffraction orders of the well-known molecular repeats in rat tail collagen (dry and wet) and cornea.

The SRS is an unusually brilliant source of X-rays; hence the flux density at the specimen is very high (ca. 4×10^{11} photons/s over a cross section of 0.6×7 mm^2 in this particular case) with a spatial resolution at the detector plane (i.e., focal spot) of ca 0.3×2 mm^2. Partly because of this, and partly because of the high sensitivity of these preparations to radiation damage, precautions had to be taken. In typical running conditions the onset of radiation damage, characterized by loss of microtubule formation, could be detected after about 20-s exposure to the X-ray beam. This problem was avoided by designing a scanning cell that ensured that the protein was never irradiated for more than 10 s; with these procedures the protein was fully functional (see Figure 2). Data reduction and evaluation procedures were carried out with the suite of programs BSL-OTOKO (Mant, Bordas, and Koch, unpublished). The procedures are essentially identical with those described in Bordas et al. (1983). Calculations of the R_G of model structures were performed by using Debye's formula (Guinier & Fournet, 1955). The model structures were constructed with either spheres or Gaussian spheres filling the model.

Results and Discussion

Radius of Gyration of Calf Brain Tubulin. Absence of Oligomers under Nonassembly Conditions. The low-angle X-ray scattering profiles of tubulin at several concentrations and temperatures in PG buffer were collected. Representative Guinier plots are shown in Figure 1. The R_G of purified calf brain tubulin is 3.10 ± 0.1 nm in the temperature range between 4 and 25 °C. There is no detectable aggregation in these conditions although interparticle interference effects manifest themselves at high concentration by the appearance of a slight undulation in the very low angle region of the traces. Substitution of GDP for GTP in the buffer, which leads to exchange of tubulin-bound nucleotide (Howard & Timasheff, 1986; Correia et al., 1987), did not induce any significant difference in the value of the R_G (± 0.1 nm). Similarly, binding of one molecule of colchicine to tubulin (Andreu & Timasheff, 1982) did not affect the measurement. However, aging the protein in PG buffer for 2–5 h at room temperature, which is known to produce a partial relaxation of secondary structure (Andreu & Timasheff, 1982) and incipient aggregation to 9S species (Prakash & Timasheff, 1982), resulted in a marked increase of R_G, to a value of 3.7–3.8 nm. This indicates that even for static measurements the short measuring times

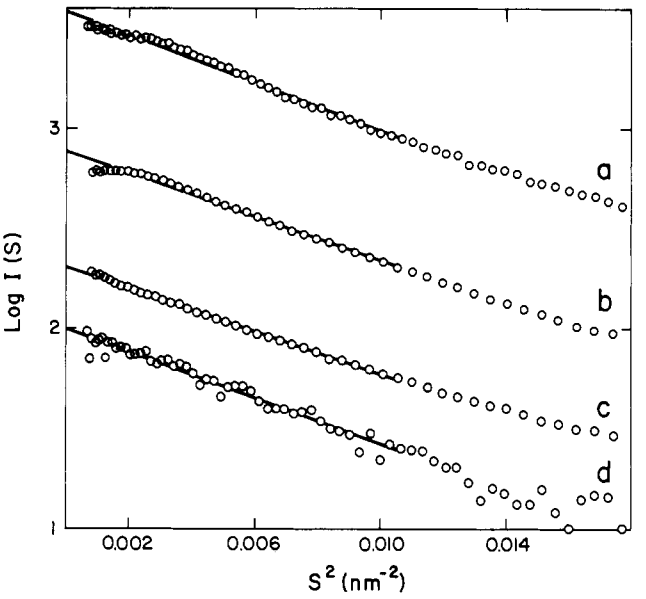

FIGURE 1: Guinier plots of purified calf brain tubulin in PG buffer at 25 °C over a range of concentrations: (a) 75 mg/mL, R_G = 3.19 nm; (b) 16 mg/mL, R_G = 3.10 nm; (c) 4 mg/mL, R_G = 3.10 nm; and (d) 2 mg/mL, R_G = 3.15 nm. The preparation used to obtain the (a) trace contained in addition 1 M sucrose and 0.5 mM $MgCl_2$.

characteristic of synchrotron radiation sources are required with this labile protein.

The R_G value of calf brain tubulin is in excellent agreement with a previous measurement with phosphocellulose-purified pig brain tubulin (3.10 ± 0.15 nm; Bordas et al., 1983). However, an important difference is the absence of detectable oligomers in the calf brain tubulin, whereas there was a significant oligomer scattering in phosphocellulose pig brain tubulin (Bordas et al., 1983). The result indicates that calf brain tubulin in PG buffer at the concentrations examined exists as a monodisperse α–β heterodimer, in agreement with previous sedimentation velocity studies (Frigon & Timasheff, 1975; Andreu & Muñoz, 1986).

A very simple model of the tubulin heterodimer consists of two touching spheres of partial specific volume 0.735 mL g^{-1} (Na & Timasheff, 1981), molecular weight 50 000 each (Ponstingl et al., 1982), and radius 2.44 nm. The calculated radius of gyration (Witz et al., 1964) for this model is 3.09 nm, in agreement with the experimental value. The relative frictional coefficient of this model, assuming a reasonable value of hydration (0.36 g of water/g of protein; Frigon & Timasheff, 1975), is $f/f_{min} = 1.20$. This is significantly smaller than the value determined by sedimentation velocity, 1.35 ± 0.05 (Frigon & Timasheff, 1975; Andreu & Muñoz, 1986), suggesting that the particles must have an irregular shape. In fact, calculations of the R_G of asymmetric models of the α–β heterodimer based on the X-ray fiber diffraction model of microtubules [Mandelkow et al., 1977; as depicted in Figure 2 of Beese et al. (1987)] yield radii of gyration of around 3.3 nm, which, within the resolution of both experiments, is compatible with our measurements.

Purified Tubulin Self-Assembly Can Be Examined by Time-Resolved X-ray Scattering. To induce tubulin assembly, assembly buffer, containing Mg^{2+} and glycerol, was substituted for PG buffer and the solution warmed. X-ray scattering profiles were obtained during the temperature rise from 4 to 37 °C. Typical results are shown in Figure 2. The scattering profiles in the cold are devoid of prominent features. The data show a certain aggregation detected by the central scattering and, possibly, some weak undulations at the positions expected

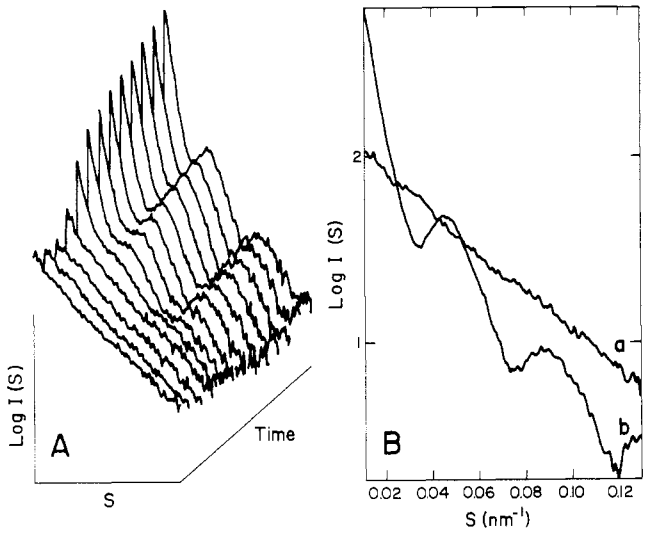

FIGURE 2: (A) Purified calf brain tubulin (4 mg/mL) in assembly buffer undergoes assembly into microtubules following a temperature jump from 4 to 37 °C. This panel shows a projection plot of the logarithm of the X-ray scattering intensities (z axis) versus the scattering vector S (x axis) and time (y axis). The time frames were collected every 10 s, but only one out of every five is shown for clarity. The moment of initiating the T-jump coincided with the end of the third profile. Notice that the cold state is free of any prominent features, while the final state shows the typical features of the X-ray traces of microtubules in solution. (B) Comparison of the initial and the final state of the data shown in panel A. The initial state does not correspond to the X-ray scattering trace expected from a uniform population of α-β heterodimer. The final state shows the typical J_0-like Bessel function behavior corresponding to the low-resolution transform of a hollow tubular structure. From the position of the subsidiary maxima it can be concluded that the mean diameter of these microtubules is similar to that of microtubules formed from microtubule protein (i.e., ca. 25.0 nm; Bordas et al., 1983), however, this does not necessarily imply that these microtubules have 13 protofilaments, as the absence of MAPs may obscure an increase in the mean diameter.

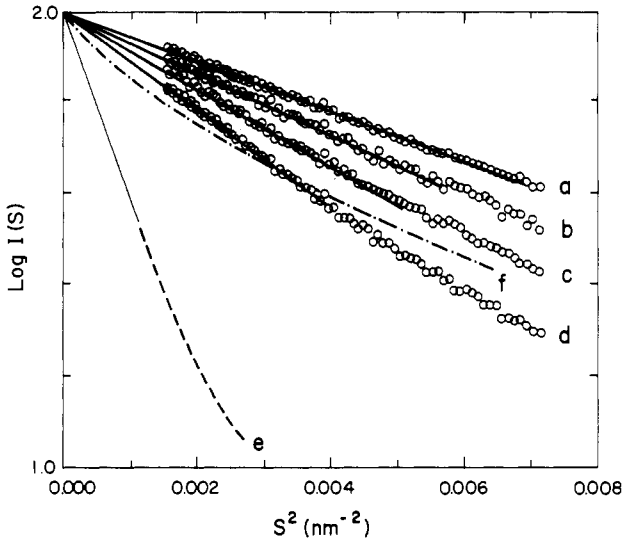

FIGURE 3: Guinier plots showing the pronounced increase in the R_G of the tubulin heterodimer with increasing concentrations of free deoxycholate. (a) Tubulin without detergent, R_G = 3.15 nm; (b) tubulin with 2.65 mM detergent, R_G = 3.46 nm; (c) tubulin with 3.13 mM detergent, R_G = 3.85 nm; (d) tubulin with 6.0 mM detergent, R_G = 4.25 nm; (e) (dashed line) Guinier plot of tubulin in 6 M guanidinium chloride; and (f) (dashed–dotted line) calculated plot for a mixture containing 60% of molecules in state a and 40% in state e. All the diagrams have been normalized to the same value at the origin to facilitate comparison.

from ringlike oligomers (Figure 2B, trace a). The average radius of gyration under these conditions is much larger than that measured in PG buffer. For example, in the case of the data shown in Figure 2B, the R_G derived from a Guinier plot of the low-angle data has a value of ca. 5.4 nm, which has to be contrasted with the 3.1-nm value obtained with PG buffer. Characteristic microtubule features (Bordas et al., 1983) gradually appear after warming the solution (Figure 2A). In the final state (Figure 2B) a marked increase in central scattering and subsidiary maxima at 0.045 and 0.087 nm^{-1} are clearly observed. This pattern corresponds to a mixture of microtubules and unassembled protein (Bordas et al., 1983). Thus, purified calf brain tubulin constitutes a simplified functional system of microtubule assembly, suitable for a more detailed characterization by time-resolved X-ray scattering. The tubulin heterodimer in Mg^{2+}-free buffer (Figure 1) can be regarded as the initial state of a cation-induced, temperature-dependent polymerization reaction leading to microtubule formation (Figure 2). This initial state of purified tubulin, in contrast to microtubule protein, does not contain detectable oligomers, which should make the kinetic analysis of the system much simpler than in the presence of MAPs. With purified tubulin, oligomers should appear transiently during the formation of polymerization nuclei.

Tubulin Heterodimer Expansion Induced by Mild Detergent Binding. In order to examine the effects of massive mild detergent binding (Andreu & Muñoz, 1986) on the structure of tubulin, the protein was equilibrated with different free deoxycholate concentrations in PG buffer, and the radii of gyration were measured. Figure 3 shows the Guinier plots of tubulin in 0, 2.5, 3.1, and 6.0 mM deoxycholate. A large increase in the slope of the linear part of the plot is observed with increasing detergent concentrations. Aggregation having been excluded (Andreu & Muñoz, 1986), this indicates a marked expansion of the protein. Notice that the linearity of the Guinier plots excludes denaturation of the protein as an explanation for the observations, as denaturation would yield a considerably more curved X-ray scattering profile. For the purpose of comparison, the dashed line in Figure 3 shows the X-ray scattering profile of tubulin unfolded in 6 M guanidine hydrochloride (Lee et al., 1973, 1978). Figure 4A displays the increase in R_G values (filled circles) compared to the detergent binding isotherm (empty circles). The increase of R_G appears to be completed before reaching detergent binding saturation. This is best seen in Figure 4B, where the fractional expansion is plotted versus fractional detergent binding; maximal expansion is produced by half-maximal binding, that is, by the first 48 ± 4 deoxycholate molecules bound per tubulin (Andreu & Muñoz, 1986). The best measurements of the radius of gyration of the saturated tubulin–deoxycholate complex yielded a value of 4.3 ± 0.1 nm, which is ca. 1.4 times the native tubulin value. This value of the R_G corresponds to the volume of the equivalent scattering sphere being twice that of the deoxycholate free molecule after corrections for the bound detergent are made.

The present X-ray scattering results, taken together with the previous sedimentation velocity and sedimentation equilibrium measurements, fully confirm the proposed expansion of the tubulin heterodimer by detergent binding (Andreu & Muñoz, 1986). The question arises as to what is the nature of the change from the compact, approximately biglobular structure of the tubulin dimer to the tubulin–deoxycholate complex. The latter cannot be simply modeled by two touching compact protein–detergent spheres (partial specific volume 0.748 mL g^{-1}; molecular weight 70000 each; Andreu & Muñoz, 1986), since this would give a calculated R_G = 3.46 nm, which is much smaller than the experimental value. Elongated or random coil models based on the frictional

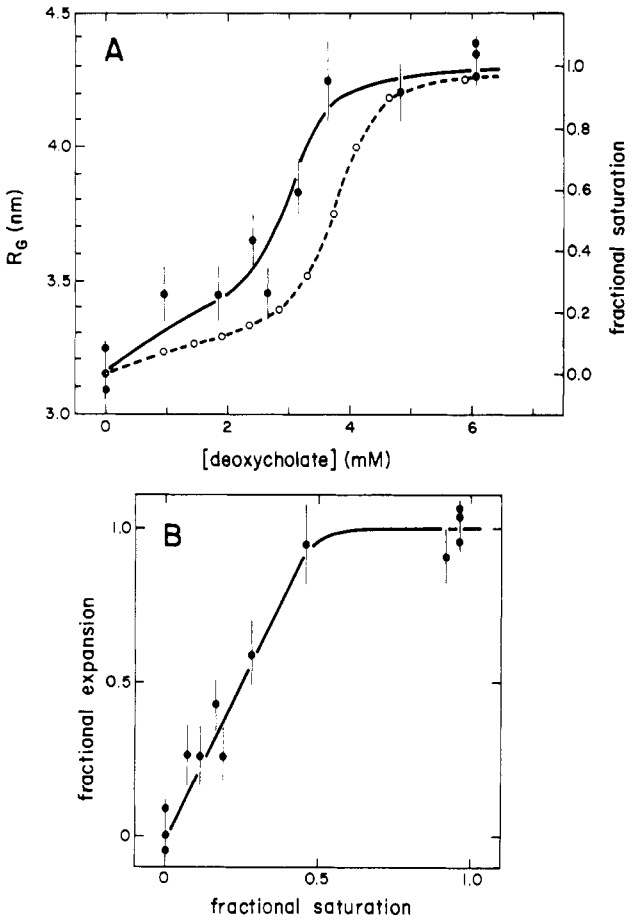

FIGURE 4: (A) Dependence of the radius of gyration (left ordinate, filled circles) and fractional saturation of deoxycholate (right ordinate, empty circles) on the concentration of detergent (abscissa). Notice that the expansion of the molecule runs somewhat ahead of the fractional saturation curve. (B) Fractional expansion of the tubulin heterodimer versus the fractional saturation with deoxycholate. The expansion of the molecule is complete at approximately 50% saturation.

coefficient value give R_G values (Tanford, 1961) of 10.4 and 10.1 nm, respectively, which are much larger than the experimental value. A simple model consists of two touching noncompact spheres having a radius of 3.40 nm each to fit the experimental R_G. This means that the protein plus detergent mass fills only 54% of the volume of the model, the rest being necessarily solvent. The relative frictional coefficient calculated for two touching spheres (Cantor & Schimmel, 1980) of 3.40-nm radius, assuming an additional hydration of 0.36 g of water/g of protein (Frigon & Timasheff, 1975), is f/f_{min} = 1.47, which is considerably smaller than the experimental value f/f_{min} = 1.86 ± 0.13 (Andreu & Muñoz, 1986). This deviation strongly suggests that the hydrodynamic tubulin–deoxycholate particle is very irregular, having extended cavities and protruding regions.

In order to compare more rigorously the radii of gyration and frictional coefficients of tubulin and the tubulin–deoxycholate complex, it is better not to assume any particular geometric model. Let us simply assume that the gross structures and hydration of tubulin and tubulin–deoxycholate are similar, the latter being an extended form of the former. This is consistent with circular dichroism measurements and controlled proteolysis, which indicate a looser structure in the tubulin–deoxycholate complex (Andreu et al., 1986). The radius of gyration of the unexpanded structure is, by definition (Tanford, 1961)

$$R_G = (\sum_i m_i r_i^2 / \sum_i m_i)^{1/2} \quad (1)$$

where r_i is the distance of each element of mass m_i to the center of mass. If the structure expands uniformly, increasing all of its dimensions by a constant factor $a \geq 1$, then the radius of gyration of the expanded structure, R'_G, is

$$R'_G = [\sum_i m_i(a^2 r_i^2) / \sum_i m_i]^{1/2} = aR_G \quad (2)$$

The translational frictional coefficient of any complex object, f, can be approximated by decomposition into an array of N identical spherical elements of frictional coefficient ζ each and application of the Kirkwood–Riseman theory, which yields (Cantor & Schimmel, 1980)

$$f/\zeta = N[1 + (1/N)\sum_i \sum_{j \neq i} (r_{ij}/R)^{-1}]^{-1} \quad (3)$$

where R is the radius of each element and r_{ij} the distance between elements i and j. If we suppose that the structure expands, increasing both r_{ij} and R by a factor a, the frictional coefficient f' of the expanded structure is

$$f'/\zeta' = f/\zeta \quad (4)$$

and

$$f' = \zeta' f/\zeta = af \quad (5)$$

Combining eq 5 and 2 provides the relationship:

$$R'_G/R_G = f'/f \quad (6)$$

For the case of tubulin and tubulin–deoxycholate three experimentally determined values are f/f_{min} = 1.35 ± 0.05, f'/f_{min} = 1.86 ± 0.13 (Andreu & Muñoz, 1986), and R_G = 3.10 ± 0.1 nm (this work). Application of formula 6 allows the calculation of the expected value of the radius of gyration of the tubulin–deoxycholate complex to be R'_G = 4.27 ± 0.35 nm, which is coincident with the experimental value of 4.3 ± 0.1 nm. This simple relationship (eq 6) does not apply when the structural elements separate without increasing their radius nor when only one of the elements detaches itself from the structure; hence, while the X-ray or the sedimentation measurements, taken independently, can be interpreted in a variety of ways, taken jointly they support an interpretation of the process whereby the molecule expands isotropically.

Thus, it can be concluded that the model of Andreu and Muñoz (1986), in which the binding of the amphiphile to previously unexposed hydrophobic regions of the molecule induces an expansion of the tubulin heterodimer, is fully supported by the X-ray scattering data.

ACKNOWLEDGMENTS

We acknowledge the support we have received from the staff of the Daresbury Laboratory and in particular that from our colleagues G. R. Jones, E. Towns-Andrews, and G. R. Mant.

Registry No. Sodium deoxycholate, 302-95-4.

REFERENCES

Andreu, J. M. (1982) *EMBO J. 1*, 1105–1110.
Andreu, J. M. (1986) *Ann. N.Y. Acad. Sci. 466*, 626–630.
Andreu, J. M., & Timasheff, S. N. (1982) *Biochemistry 21*, 6465–6476.
Andreu, J. M., & Muñoz, J. A. (1986) *Biochemistry 25*, 5220–5230.
Andreu, J. M., Gorbounoff, M. J., Lee, J. C., & Timasheff, S. N. (1984) *Biochemistry 23*, 1742–1752.
Andreu, J. M., de la Torre, J., & Carrascosa, J. L. (1986) *Biochemistry 25*, 5230–5239.
Beese, L., Stubbs, G., & Cohen, C. (1987) *J. Mol. Biol. 194*, 257–264.

Bordas, J., Koch, M. H. J., Clout, P. N., Dorrington, E., Boulin, C., & Gabriel, A. (1980) *J. Phys. E: Sci. Instrum., 13*, 938-944.
Bordas, J., Mandelkow, E. M., & Mandelkow, F. (1983) *J. Mol. Biol. 164*, 89-135.
Cantor, C. R., & Schimmel, P. R. (1980) *Biophysical Chemistry*, Freeman, San Francisco.
Correia, J. J., Baty, L. T., & Williams, R. C., Jr. (1987) *J. Biol. Chem. 262*, 1727-1728.
Frigon, R. P., & Timasheff, S. N. (1975) *Biochemistry 14*, 4559-4566.
Guinier, A., & Fournet, G. (1955) *Small Angle Scattering of X-rays*, Wiley, New York.
Howard, W. D., & Timasheff, S. N. (1986) *Biochemistry 25*, 8292-8300.
Lee, J. C. (1982) *Methods Cell Biol. 24*, 9-30.
Lee, J. C., & Timasheff, S. N. (1975) *Biochemistry 14*, 5183-5187.
Lee, J. C., & Timasheff, S. N. (1977) *Biochemistry 25*, 1754-1764.
Lee, J. C., Frigon, R. P., & Timasheff, S. N. (1973) *J. Biol. Chem. 256*, 7193-7201.
Lee, J. C., Corfman, D., Frigon, R. P., & Timasheff, S. N. (1978) *Arch. Biochem. Biophys. 185*, 4-14.
Lewis, R. A., Sumner, I., Berry, A., Bordas, J., Gabriel, A., Mant, G., Parker, B., Roberts, K., & Worgan, J. S. (1988) *Nucl. Instrum. Methods Phys. Res. A273*, 773-777.
Mandelkow, E. (1986) *Methods Enzymol. 134*, 633-657.
Mandelkow, E., & Bordas, J. (1986) *Methods Enzymol. 134*, 657-676.
Mandelkow, E., Thomas, J., & Cohen, C. (1977) *Proc. Natl. Acad. Sci. U.S.A 74*, 3370-3374.
Mandelkow, E. M., Harmsen, A., Mandelkow, E., & Bordas, J. (1980) *Nature 287*, 595-599.
Mandelkow, E. M., Lange, G., Jagla, A., Span, U., & Mandelkow, E. (1988) *EMBO J. 7*, 357-365.
Na, G. C., & Timasheff, S. N. (1981a) *J. Mol. Biol. 151*, 165-178.
Na, G. C., & Timasheff, S. N. (1981b) *Methods Enzymol. 85*, 393-408.
Ponstingl, H., Krauhs, E., Little, M., Kempf, T., Hofer-Warbinek, R., & Ade, W. (1982) *Cold Spring Harbor Symp. Quant. Biol. 46*, 191-197.
Prakash, V., & Timasheff, S. N. (1982) *J. Mol. Biol. 160*, 499-515.
Spann, U., Renner, W., Mandelkow, E. M., Bordas, J., & Mandelkow, E. (1987) *Biochemistry 26*, 1123-1132.
Tanford, S. N. (1961) *Physical Chemistry of Macromolecules*, Wiley, New York.
Timasheff, S. N. (1981) in *Molecular Approaches to Gene Expression and Protein Structure* (Siddiqui, M. A. Q., Krauskopf, M., & Weisbach, H., Eds.) pp 245-285, Academic Press.
Towns-Andrews, E., Berry, A., Bordas, J., Mant, G. R., Murray, P. K., Roberts, K., Sumner, I., Worgan, J. S., & Lewis, R. (1989) *Rev. Sci. Instrum.* (in press).
Weisenberg, R. C., Borisy, G. G., & Taylor, E. (1986) *Biochemistry 7*, 4466-4479.
Witz, J., Timasheff, S. N., & Luzzati, V. (1964) *J. Am. Chem. Soc. 86*, 168-173.

Translation of the Human C3b/C4b Receptor mRNA in a Cell-Free System and by *Xenopus* Oocytes

V. Kumar, T. Farries, J. Swierkoz, and J. P. Atkinson*

Howard Hughes Medical Institute Laboratories and Department of Medicine, Division of Rheumatology, Washington University School of Medicine, St. Louis, Missouri 63110

Received September 15, 1988; Revised Manuscript Received December 20, 1988

ABSTRACT: The C3b/C4b complement receptor (CR1) is a large, single-chain integral membrane glycoprotein present on erythrocytes, leukocytes, glomerular podocytes, and splenic dendritic-reticular cells that mediates the binding of complement-coated particles and immune complexes. CR1 is unusual in that it is polymorphic in size with the four allelic variants having molecular weights of 190 000, 220 000, 250 000, and 280 000 (SDS-PAGE, reducing conditions). The in vitro translation of the common (M_r 220 000) allelic variant CR1 has been achieved by using mRNA in lysates of rabbit reticulocytes and in *Xenopus* oocytes. HL-60, a promyelocytic human leukemic cell line, was treated with DMSO to induce differentiation and synthesis of CR1. Poly(A+) RNA was purified from these cells by column chromatography on oligo(dT)-cellulose. In the rabbit reticulocyte system, no CR1 was detected unless the translation mixture was denatured. In the presence of methylmercuric hydroxide, the CR1 translation product, unlike most translation products, had the same molecular weight in gel electrophoresis as the high-mannose-containing pro-CR1 and was 15-20K larger than nonglycosylated CR1. This suggests that a cotranslational modification of CR1 structure occurs, probably involving a proteolytic cleavage event. When poly(A+) RNA was translated in *Xenopus* oocytes, CR1 could be detected by treatment of oocytes with anti-CR1 monoclonal antibody followed by fluorescein-conjugated goat anti-mouse IgG. CR1 was diffusely distributed but preferentially localized to the vegetal surface. The molecular weight of this product, identified in immunoprecipitates of lysates of [^{35}S]methionine-labeled oocytes, was identical with that of CR1 of HL-60.

Complement and receptors for complement activation fragments play an important role in the identification and processing of microbes (Fearon & Wong, 1983; Ross & Medof, 1985; Reid, 1986; Muller-Eberhard, 1988). The third component of complement (C3) occupies a central place in this host defense system. During complement activation, C3

0006-2960/89/0428-4040$01.50/0