APPENDIX

Hydrodynamic Analysis of Tubulin Dimer and Double Rings

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This Appendix describes rigorous calculations of the sedimentation coefficients of the bead models of tubulin monomer, dimer and double rings employed in the X-ray scattering analysis of GDP-tubulin rings (Díaz et al., 1994). In their elegant study of magnesium-induced tubulin self-association, Frigon & Timasheff (1975) carried out an early hydrodynamic analysis of the tubulin dimer, analyzed in terms of a hydrated anisometric ellipsoid, and of the 42 S double-ring end product. The tubulin double ring structure was simplified to a single plane polygon, of dimensions estimated by electron microscopy, having a frictional unit at each vertex with the experimental frictional coefficient and molecular weight of a tubulin dimer. The hydrodynamic calculation was based on the Kirkwood (1954) formula, well known to be a simple approximation. Zwanitz (1966) showed that for rings with many units this formula underestimates the frictional coefficient (or overestimates the sedimentation coefficient) up to 8%. The conclusion that tubulin rings are made of 26 ± 2 tubulin dimers (Frigon & Timasheff, 1975) is compatible with the X-ray scattering analysis, however, accurate discrimination between models with different number of monomers per ring was not possible. In the present analysis the hydrodynamics of the tubulin dimer is characterized based on the X-ray scattering model and its experimental sedimentation coefficient, and the approximate frictional coefficient of the tubulin monomer is extracted. Then the sedimentation coefficients of the pertinent X-ray double ring models are calculated with rigorous hydrodynamic theory, employing the molecular weight and frictional coefficient of the monomer and the double ring dimensions determined by X-ray scattering. The results strongly support the X-ray scattering analysis and allow selection of the number of tubulin subunits in each ring.

(a) Hydrodynamic computer modelling and experimental parameters

The calculation of sedimentation coefficients and other solution properties has been carried out employing the most advanced versions of the hydrodynamic theory and computer programs for multi-subunit structures (García de la Torre & Bloomfield,
Table A1

Hydrodynamic properties of tubulin dimer, monomer and double ring bead models

<table>
<thead>
<tr>
<th>Bead radius (nm)</th>
<th>$s_{20,w}^{1}$ (S)</th>
<th>$s_{20,w}^{2}$ (S)</th>
<th>$f_1 \times 10^8$ (ergs units)</th>
<th>Number of monomers per ring</th>
<th>$s_{20,w}^{2}$ (S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2</td>
<td>6.49</td>
<td>4.16</td>
<td>5.80</td>
<td>399</td>
<td>42.9</td>
</tr>
<tr>
<td>1.4</td>
<td>6.47</td>
<td>3.91</td>
<td>6.18</td>
<td>394</td>
<td>42.4</td>
</tr>
<tr>
<td>1.6</td>
<td>5.89</td>
<td>3.70</td>
<td>6.52</td>
<td>389</td>
<td>41.9</td>
</tr>
<tr>
<td>1.7</td>
<td>5.55</td>
<td>3.60</td>
<td>6.69</td>
<td>387</td>
<td>41.6</td>
</tr>
<tr>
<td>1.8</td>
<td>5.63</td>
<td>3.61</td>
<td>6.87</td>
<td>385</td>
<td>41.4</td>
</tr>
<tr>
<td>1.9</td>
<td>5.51</td>
<td>3.42</td>
<td>7.05</td>
<td>383</td>
<td>41.2</td>
</tr>
</tbody>
</table>

† Sedimentation coefficient.
‡ Frictional coefficient.

1980; García de la Torre, 1989; 1992). For the detailed structure of the non-globular tubulin units, the Cartesian coordinates of the small spherical beads, as obtained from the X-ray scattering simulation software, were used directly in the hydrodynamics programs.

The tubulin dimer has a sedimentation coefficient $s_{20,w} = 5.8$ S, a partial specific volume of $0.736 \text{ ml g}^{-1}$, and molecular mass $M = 2 \times 50,000$ (Lee et al., 1973; Frigon & Timasheff, 1975; Howard & Timasheff, 1986; Shearwin & Timasheff, 1992; Rivas & Andreu, unpublished results), experimentally determined employing the same purified protein under conditions similar to the X-ray scattering study. This gives a frictional coefficient $f = 83 \times 10^{-8}$ ergs units. Similarly, the sedimentation coefficient of tubulin rings is $s_{20,w} = 42 \pm 2$ S (Frigon & Timasheff, 1975), a value shown to be independent of having GDP or CTP at the exchangeable nucleotide site of the protein and refined to $42 \pm 1$ S with colchicine-ligated tubulin (Howard & Timasheff, 1986; Shearwin & Timasheff, 1992).

(b) Tubulin dimer and monomer

The hydrodynamic dimer model was obtained from the X-ray scattering model of tubulin rings (Diaz et al., 1994), by taking a set of 42 spheres corresponding to two adjacent monomeric units, each made of 21 spheres of radius $r = 1.2$ nm, with the above molecular mass. The calculated sedimentation coefficient for the dimer is $s_{20,w} = 6.5$ S, which is 12% larger than the experimental value of 5.8 S. A slight overestimation such as this can be well explained in terms of hydration. Estimating hydration as about 0.36 g water per g tubulin (Frigon & Timasheff, 1975) amounts roughly to an increase in the main dimensions of the hydrated particle with respect to the anhydrous one of about 12%. In models of arbitrary shape made up of beads, a possible way to account for hydration effects is through the radius of the beads. This parameter can be increased beyond the values that the beads in the model would have if they were just touching their neighbours, while keeping the molecular mass constant. Actually, the theory and computer programs employed embody the hydrodynamic interaction tensor that accounts for this possibility (Rotne & Prager, 1970; Yamakawa, 1970). This way, in a series of calculations, the bead radius was increased beyond the 1.2 nm value used in scattering modelling. For an appreciably larger value such as, for instance, $r = 1.6$ nm, the increase in the main dimensions of the particle would be $2 \times (1.6 - 1.2)$ nm = 0.8 nm. From the X-ray scattering model structure (the size of the monomer is roughly 4 nm × 7 nm × 8 nm), the relative size increase averaged over the three inertial axes would be 13%, which is compatible with the above estimate from hydration.

The results of the series of calculations for the dimer are shown in Table A1. We note that the experimental sedimentation coefficient value can be accurately reproduced by the calculations for $1.6 < r < 1.7$ nm, which is a quite reasonable choice as discussed above. Allowing for an admissible error in the sedimentation coefficient, for example, taking as experimental value 5.6 S (Andreu & Muñoz, 1986), would yield a larger but not unreasonable value, $1.8 < r < 1.9$ nm, for the bead radius of the model. Therefore, it is possible to simulate the experimental sedimentation coefficient of the dimer using the very same X-ray scattering model in the hydrodynamic analysis.

The hydrodynamic properties of the monomer can be estimated by a similar calculation, for a model of 21 beads for the subunit taken from the X-ray model of the whole double ring. The values obtained for the friction and sedimentation coefficient ($M = 50,000$) are listed in Table A1. The experimental sedimentation coefficient of the tubulin monomers is not presently available. Though it has been assumed for the purpose of the hydrodynamic analysis that the tubulin dimer has similar shape in its unassembled and assembled states, it may also be considered that the structure of tubulin should differ in the different aggregation states. Employing a tubulin dimer taken from
microtubule models, instead of ring models, gave a very small change in the calculated sedimentation coefficient. Therefore, these differences have been ignored in the first approximation. A more exact analysis would require more detailed X-ray modelling of the tubulin monomer in its different association states.

(c) Tubulin double rings

The tubulin double rings have been modelled for hydrodynamic purposes by regular concentric polygons of \( n_1 \) and \( n_2 \) vertices, which are occupied by monomeric units having molecular mass 55,000 Da and friction coefficient \( f_1 \). For the latter parameter any of the plausible values estimated (listed in Table A1) can be taken. The radii of the outer and inner rings are constrained to the respective values 21.8 nm and 16.2 nm, determined by X-ray scattering (Díaz et al., 1994), and calculation of the Cartesian coordinates of the vertices of the polygons is trivial. This has been done with several choices for the numbers of monomers in each ring, \( n_1 + n_2 \), namely 30 + 22, 32 + 24, 34 + 26 and 36 + 28 (corresponding to 26, 28, 30 and 32 tubulin dimers). The calculated sedimentation coefficients of the double rings are shown in Table A1. The values obtained for the case with 32 + 24 subunits are in excellent agreement with the experimental data, \( s_{20,w} = 42 \pm 1 \) S. The calculated coefficient of rings is rather insensitive to the choice of \( f_1 \), in contrast to the case of the dimer. Particularly, for the same values of \( f_1 = (6.52-6.69) \times 10^{-8} \) cgs units that reproduce the experimental \( s_{20,w} \) of the dimer, we calculate for the double ring \( s_{20,w} = 41.9 \) to 41.6 S, which is coincident with the experimental result. For the other choices of \( n_1 + n_2 \), it can be observed that, regardless of the tabulated value of \( f_1 \), the calculated values differ from the experimental result beyond the error of the latter.

In conclusion, X-ray scattering analysis of GDP-tubulin double rings plus rigorous hydrodynamic treatment gives a fully accurate description of the experimental sedimentation values, and strongly favours the 32 + 24 monomer double ring model.

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References


