A STUDY OF MICROTUBULE STRUCTURES IN SOLUTION BY SMALL-ANGLE X-RAY SCATTERING

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Received 10 October 1977

1. Introduction

One of the universal components of eukaryotic cells are microtubules, hollow cylindrical structures whose length considerably exceeds their diameter. According to electron microscopy studies of fixed samples, the diameter of microtubules is similar virtually in all the studied cells and is 230–270 Å (e.g. [1,2]) and the walls are about 40 Å thick. The main structural component of microtubules is the protein tubulin, which under appropriate conditions (removal of Ca^{2+} ions, presence of GTP and temp. 37°C) is capable of forming microtubules in solution. From electron microscopy data of negatively stained preparations, the dimensions of microtubules determined in solution coincide with those obtained from investigation of sections. It is quite possible that during the preparation of the samples for electron microscopy, the structure of microtubules can be deformed owing to fixation, staining and (or) dehydration of the preparation. Therefore it is important to determine the microtubule parameters directly in solution.

This paper presents the results of investigation of the microtubule structure by small-angle X-ray scattering which, as is known, is a direct and the most sensitive method of investigating macromolecule structure in solution. The experimental curves were treated according to the method developed by one of us [3,4] using the Hankel transform of the diffuse scattering indicatrix for long non-uniform cylinders. This method provides information on the radial distribution of the electron density in the cylinder and it has been already used for the study of the structure of tobacco mosaic virus [5] and flagella from *Salmonella* [6].

2. Materials and methods

Tubulin was obtained from cattle brain by polymerization–depolymerization [7]. The brain was homogenized in a buffer solution A containing 50 mM inidazole, 50 mM KCl and 0.5 mM $MgCl_2$, pH 6.7. The homogenate was clarified by centrifugation at 10 000–15 000 × g for 30 min and GTP was added to the supernatant to 0.1 mM final concentration and centrifugation was repeated at 10 000 × g for 30 min.

Homogenization and clarification were done at 2–4°C. The supernatant obtained after the second clarification was diluted two-fold by buffer A containing, in addition, 8 M glycerin and 2 mM EDTA and was incubated for 2 h at 37°C. The obtained microtubules were precipitated by centrifugation at 100 000 × g for 60 min (rotor temp. 25°C). The microtubule precipitates were suspended in buffer A with 4 M glycerin and 1 mM EDTA (1 ml buffer/15 ml initial clarified homogenate) and stored at 4°C for not more than 2 weeks.

X-Ray diffuse scattering was measured in a small-angle Kratky camera independently at the Zentralinstitut für Molekularbiologie, Akademie der Wissenschaften der DDR, and at the Institute of Protein Research, Academy of Sciences of the USSR; the obtained scattering indicatrices were essentially the same. The high stability 'Geigerflex' D-9C generator (Rigaku Denki) ensuring the intensity stability within 1% was used in the X-ray apparatus of the Institute of Protein Research described [8]. The scattered emission was registered by a scintillation counter, the CuK_α line was discriminated by a nickel β-filter combined with an amplitude discriminator. Measurements were performed at two intervals of angles, from $\mu = 0.005$ Å^{-1} to $\mu = 0.08$ Å^{-1} and from $\mu = 0.01$ Å^{-1} to $\mu = 0.17$ Å^{-1}. The width of the collimation and collector slits were 60 μM and 120 μm, respectively, for the first interval, and 150 μm and 240 μm for the second interval. Intensities of scattering from the solution and solvent placed in a cuvette with two identical compartments 1.1 mm wide were alternatively measured at each angle. 10^5–10^6 impulses were counted for each measured point. The collimation correction for the slit height was made according to [9].

The function of the radial distribution of the electron density $\rho(r)$ was calculated by the equation

$$\rho(r) = \frac{1}{2\pi^{3/2}} \int_{\mu_{min}}^{\mu_{max}} (\pm \sqrt{I(\mu)\mu})\, J_o(\mu r)\mu d\mu \qquad (1)$$

where $I(\mu)$ is the experimental intensity of scattering measured in the interval from μ_{min} to μ_{max} ($\mu = \frac{4\pi}{\lambda}\sin\theta$, λ is the wavelength of X-rays, 2θ is the scattering angle); $J_o(\mu r)$ is the Bessel function of the zero order. The resolving power of the function $\rho(r)$ was increased by extrapolating the experimental curve $I(\mu)$ in the region of the smallest angles (up to $\mu = 0.001$ Å^{-1}) according to the technique described in [5]. To suppress the effects appearing on the $\rho(r)$ curve owing to a truncation of the experimental scattering indicatrix at μ_{max}, the scattering intensity was multiplied by the 'temperature factor' $e^{-\alpha\mu^2}$, the factor α was chosen so as to decrease the intensity at μ_{max} by one order. At calculation of the scattering amplitude $\pm\sqrt{I(\mu)\mu}$ an alternating order of signs (+–+–) was used, but to control the reliability of the obtained distribution function the variant (+–++) was also considered.

3. Results and discussion

Figure 1 represents the obtained experimental curve of microtubule scattering in solution. The dotted lines denote the region of extrapolation to the smallest angles and the region of correction of the experimental scattering indicatrix when the sign of the scattering amplitude is changed. Figure 2 represents the result of the integral transform according to eq. (1). The solid line denotes the distribution function at the alternating order of signs of the scattering amplitude, the dotted line corresponds to a less reliable variant (+–++) chosen because of the low resolution of the last maximum on the experimental curve.

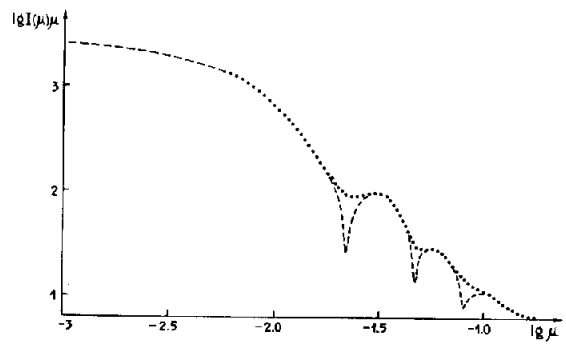

Fig.1. Experimental curve of scattering by microtubules in solution (. . .). Extrapolation to the smallest angles and correction of the scattering indicatrix when the sign of the scattering amplitude is changed (- - -).

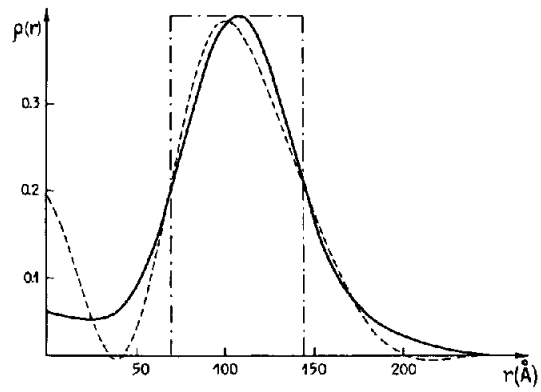

Fig.2. Function of electron density distribution of the microtubule cross section. (——) Function corresponding to the alternating order of signs (+–+–) of the scattering amplitude. (- - -) Function corresponding to a less reliable variant of sign alternation (+–++) of the scattering amplitude. (- . -) Cylinder with the internal and external diameter of 140 Å and 290 Å, respectively.

As seen in fig.2, both considered variants lead to virtually the same structural parameters characterizing microtubules in solution; both functions of the radial distribution of the electron density testify to the presence of an internal cavity in the cylinder with a diameter of ~ 140 Å (this cavity is especially noticeable in the first, more reliable, variant) and results in an external diameter of the cylinder of ~ 290 Å. This corresponds to the average cylinder radius of ~ 110 Å and a wall thickness of ~ 75 Å.

The obtained value of the average cylinder radius coincides well with the data of [10] based on the X-ray diffraction of oriented microtubule samples. However the thickness of the microtubule walls as well as their external diameter is somewhat larger than evidenced by electron microscopic data. This can be explained in two ways. First, protein may be specifically or non-specifically sorbed on the microtubule surface which results in thickening of microtubule walls. Thus, in [11,12] it is shown that minor components of tubulin obtained by the method of polymerization–depolimerization form a filamentous material on the microtubule surface. Secondly, it cannot be excluded that dehydration of microtubules takes place at processing of electron microscopic preparations which leads to a decrease of their dimensions.

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