Analysis of models of irregular shape by solution X-ray scattering: the case of the 50S ribosomal subunit from *E. coli*

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Structural models of biological macromolecules can be tested by comparing calculated and experimental solution scattering curves. We have developed an approach for computing scattering shape functions at medium resolution from models proposed on the basis of other techniques such as electron microscopy. We present the results obtained with the 50S ribosomal subunit from *Escherichia coli*; two models are considered, one proposed by Lake (1976), the other one by Tischendorf *et al.* (1975). Although the two models are similar in many respects, their scattering shape functions are significantly different. The comparison with the experimental scattering curve allows us to check the scale of the models and, after scaling, to quantitate the agreement between the observed and the calculated curves. Finally, it can provide a starting point for the structural interpretation of the X-ray data.

Key words: contrast variation/low resolution model calculations/ribosome structure/synchrotron radiation

Introduction

X-ray scattering is one of the most powerful techniques available to study the structure of biological macromolecules in solution (Luzzati and Tardieu, 1980). However, there is no direct way to extract information relevant to the three-dimensional (3-D) structure from solution scattering data. In fact, a variety of models could have the same scattering curve, especially when errors are taken into account. One can only build a model compatible with the data and restrict the range of acceptable solutions by making use of all the relevant experimental data obtained by other techniques. Of such techniques electron microscopy is particularly informative with respect to the overall shape and symmetry (or lack of it) of macromolecules whose dimensions are of the order of hundreds of angstrom or more. Such studies may lead to 3-D model which accounts for all the different views identified on the electron micrographs. Making use of such models for the interpretation of solution scattering data requires the calculation of scattering patterns; in the case of an irregular shape, this is a delicate problem which has already been approached in various ways.

A study of the 50S ribosomal subunit from *Escherichia coli* that we had recently undertaken prompted us to develop a method to calculate scattering patterns. A set of scattering curves had been recorded as a function of solvent density and the analysis of these curves had yielded several parameters as well as a good approximation of the scattering shape function at low resolution (Vachette, 1979). In addition, plaster copies

of two models proposed on the basis of electron micrographs were available to us (see Figure 1): model I proposed by Lake (1976) and model II by Tischendorf *et al.* (1975), (see also Stöffler and Wittmann, 1977). We were able to determine a scale for each model from our experimental data; in addition, differences in the shapes of the models appeared to result in significant differences in the calculated scattering patterns even at low resolution ($s = 2\sin\theta/\lambda < 0.016 \text{ \AA}^{-1}$). Thus, the comparison between the calculated and experimental curves provides a critical test of models proposed on the basis of other techniques; it can also provide a starting point for a structural interpretation of solution X-ray scattering (SXRS) data by refining the particle shape and introducing the fluctuations of density within the particle.

Results

Model calculations

There is no rigorous way to calculate the scattering pattern of a solid model which is not described by analytical functions; a number of approximate procedures have been developed. The method we designed is best suited to deal with low resolution models described by surface digitalisation. Let $i(s)$ and $p(r)$ be the spherical average of the scattered intensity and the auto-correlation function of the electron density distribution of the particle, respectively. These functions are related by Fourier transformation:

$$s i(s) = 2 \int_0^\infty r p(r) \sin(2\pi rs) ds \quad (1)$$

The method makes use of the following property of the auto-correlation function: $p(r)$ measures the common volume between the particle and its "ghost" translated by r (Guinier and Fournet, 1955). We operate with rectangular coordinates x, y, z . Let us consider a square grid of side a in the (x, y) plane and orient the model so that any line parallel to the z -axis crosses the surface only twice (if there were no such orientations for a given particle, the method could still be used with minor modifications). Let us draw the line parallel to the z -axis at a point (x, y) of the grid: z_1 and z_2 are the heights at which this line crosses the lower and upper surfaces of the particle. In this way the volume can be filled by adjacent square prisms parallel to the z -axis and the model can be described by a series of pairs $z_1(x, y), z_2(x, y)$. The principle of the calculation is to translate the particle in x, y , and z directions by an integral number n_x, n_y , and n_z of elementary translations equal to a , and to calculate the common volume $V(r)$ of the particle and its translated ghost. (If the z_1 and z_2 values were integral multiples of a this would be strictly equivalent to filling the volume with cubes and counting the number of common cubes.) The length of the translation vector r is:

$$r = a \sqrt{n_x^2 + n_y^2 + n_z^2} \quad (2)$$

The common volume is then histogrammed as a function of r , with a chosen width for the histogram classes. The

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Fig. 1. Photographs of the models proposed for the 50S ribosomal subunit from *E. coli* by Lake (1976) for model I (1a) and Tischendorf *et al.* (1975) for model II (1b). Dimensions in insets are as given by the authors.

spherical average is obtained by dividing the content of each class by the corresponding number of translations. When this process is completed the intensity curve is derived using equation (1).

The method was tested in the case of a cylinder. The comparison of the intensity curves computed by this method, at a resolution similar to the model calculations, and semi-analytically is represented in Figure 2.

Analysis of the models

The first step of our model analysis was to determine the intensity curve corresponding to the shape of the 50S subunit. The intensity curves recorded as a function of contrast $\Delta\rho_0$ (see **Materials and methods**) can be written (Stuhrmann and Kirste, 1965; Luzzati and Tardieu, 1980):

$$i(s, \rho_0) = \Delta\rho_0^2 i_v(s) + \Delta\rho_0 i_{qv}(s) + i_q(s) \quad (3)$$

where $i_v(s)$ corresponds to the shape of the particle and $i_q(s)$ to the inner fluctuations of density; $i_{qv}(s)$ is a cross term. Once a series of curves has been recorded at different values of ρ_0 , $i_v(s)$, $i_q(s)$ and $i_{qv}(s)$ can be determined. In fact, the 50S ribosomal subunit has a high average electron density $\bar{\rho} = 0.455 \text{ el}/\text{\AA}^3$ (see Figure 3). In the absence of sucrose, the density contrast with the solvent is much higher than the electron density fluctuations inside the particle and the contribution of the internal structure to small-angle scattering becomes negligibly small out to $s = 0.016 \text{ \AA}^{-1}$. Therefore, we performed the comparisons between calculated and experimental curves using the curve recorded in water instead of an extrapolated shape function. This curve is similar to those reported by Hill *et al.* (1969a), Tolbert (1971) and Serdyuk (1975); moreover the first shoulder, occurring at $s = 0.011 \text{ \AA}^{-1}$, is close to the one present in the low resolution shape function determined by Stuhrmann *et al.* (1976, 1977).

The scale of each model was determined by setting the volume of the model equal to the volume of the particle provided by SXRS experiments. This SXRS volume was obtained from the variation of the square root of the normalised intensity at the origin, $\sqrt{i_n(0, \rho_0)/c_e}$, as a function of the elec-

Fig. 2. Intensity curves for a circular cylinder of volume $2.5 \cdot 10^6 \text{ \AA}^3$ and height $200 \text{ \AA}$. —, calculated using a semi-analytical method: the common volume to a cylinder and its translated ghost can be determined analytically for any translation vector $\mathbf{r}$; spherical averaging is numerically performed. ...., calculated with the program described in the text, using a square grid of $10 \text{ \AA}$ side. The axis of the cylinder was parallel to the x-axis. The scattering angle is defined by $s = 2\sin\theta/\lambda$.

tron density of the solvent, ρ_0 . A detailed analysis of this variation has already been presented elsewhere (Tardieu *et al.*, 1981). The values recorded as a function of sucrose concentration can be fitted by a straight line (see Figure 3) and the hydrated volume V is derived from the slope of this line according to the following expression (Sardet *et al.*, 1976; Luzzati and Tardieu, 1980):

$$\sqrt{i_n(0, \rho_0)/c_e} = \frac{V}{\sqrt{m}} (\bar{\rho} - \rho_0) \quad (4)$$

where m is the number of electrons of the dry particle and $\bar{\rho}$

the mean electron density of the hydrated particle. V is found to be equal to $2.50 \pm 0.05 \cdot 10^6 \text{ \AA}^3$, which corresponds to a hydration of $0.37 \pm 0.2 \text{ g H}_2\text{O/g particle}$ (Tardieu *et al.*, 1981). This volume was considered to be the relevant volume for subsequent calculations as the electron microscopic models represent the external envelope of the particle. The correspondence between cm and $\text{\AA}$ for both models was then adjusted, so that the volume of the model becomes equal to V ; 5 mm turn out to correspond to 11.0 $\text{\AA}$ in model I and 4 mm to 9.0 $\text{\AA}$ in model II. In the case of model I, this scaling procedure led to a linear scale 15% less than the published one. The scale found for model II is in agreement with the published dimensions of 160 $\text{\AA} \times 200 \text{ \AA} \times 230 \text{ \AA}$ if these values represent the maximum dimensions in the x , y , and z directions as indicated in Figure 1.

Fig. 3. Plot of $\sqrt{I_n(0, \rho_0)/C_e}$ as a function of ρ_0 . The correlation coefficient of the linear regression is 0.969.

We then calculated the scattering patterns of the two models as indicated above. Since the model measurements were made on square grids of 5 and 4 mm, respectively, the edges of the lattice used for the calculations were 11 and 9 $\text{\AA}$. After scaling the curves, some parameters such as the radius of gyration and the greatest diameter of the particle were readily determined from the distance distribution function $p(r)$. The radii of gyration of the two models were between 77 $\text{\AA}$ and 78 $\text{\AA}$ as compared to $75 \pm 0.5 \text{ \AA}$ for the radius of gyration in the absence of sucrose. The maximal chords were ~ 245 (model I) and 240 $\text{\AA}$ (model II), to be compared with $255 \pm 20 \text{ \AA}$ experimentally determined (Vachette, 1979). This agreement is excellent, given the resolution of the calculations and the models themselves.

The calculated scattering patterns are compared to the experimental data in Figure 4. The calculated curve of model I is in good agreement with the experimental data out to $s = 0.016 \text{ \AA}^{-1}$. The position and intensity of the first shoulder are practically identical. But, in the case of model II this broad shoulder is split into two. In order to quantify the differences between experimental and calculated curves, an R factor was defined as follows:

$$R = \frac{\sum_{s_1}^{s_2} s^2 [I_{\text{calc}} - I_{\text{obs}}]}{\sum_{s_1}^{s_2} s^2 [I_{\text{obs}}]} \quad (5)$$

The s^2 weighing has been chosen because of the overall decrease of intensity in the angular range of interest. In the range $0.0054 \text{ \AA}^{-1} \leq s \leq 0.0163 \text{ \AA}^{-1}$ this factor was found to be 12% for model I and 27% for model II. A second maximum is found at $s \geq 0.02 \text{ \AA}^{-1}$ for model I, but it is shifted with respect to the one observed on the experimental curve.

Finally, the larger intensity which is observed in the experimental curve for $s \geq 0.02 \text{ \AA}^{-1}$ can be ascribed to internal fluctuations of electron density whose contribution to scatter-

Fig. 4. Comparison of the experimental data with the curves calculated for model I (4a) and model II (4b) after scaling (see text for details).

ing becomes significant (note that the intensity in this angular range is three orders of magnitude lower than the intensity at the origin. Furthermore, because of the grid size, the calculation is no longer accurate in this region).

Discussion

We have developed an approach to evaluate different structural models proposed for one particle, and applied it to the 50S ribosomal subunit from *E. coli*. This approach is based on the comparison of the scattering shape functions calculated for each model with an experimental X-ray scattering curve. Similar comparisons on the same particle were presented by Tolbert several years ago (1971). However, that study remained purely qualitative, while the electron microscopic models to be tested were rather different. More detailed and similar models have been proposed since; the two models we consider, although clearly distinct, appear to be largely two different interpretations of rather similar images. Moreover, since 1971 solution X-ray scattering data have been greatly improved by technological developments, so that a more quantitative analysis can now be achieved.

The approach involves two major steps, the determination of the X-ray scattering shape function ($i_v(s)$ function) of the particle and the computation of the scattering pattern of the model. For a chemically homogeneous particle (e.g., a protein) one can generally assume that the electron density distribution is uniform within the particle; in that case the scattering is essentially due to the shape of the particle, with a negligible contribution from density fluctuations. $i_v(s)$ is then directly given by the experiment. In the case of complex particles with chemically different components (ribosomes, lipoproteins, viruses, etc.) the internal fluctuations of electron density cannot generally be neglected and a complete contrast variation study must be performed to determine the function $i_v(s)$ (Luzzati and Tardieu, 1980). In the case of the 50S ribosomal subunit, this was achieved by Stuhmann *et al.* with neutron scattering (Stuhmann *et al.*, 1976; Stuhmann *et al.*, 1977); yet the function $i_v(s)$ could only be obtained with sufficient accuracy at very low resolution. These authors were able, however, using spherical harmonics, to propose a model at 100 Å resolution which shows some typical features common to both electron microscopic models we considered.

In his work, Tolbert (1971) recorded four scattering curves at different contrast using sucrose and found no significant variation of the radius of gyration as a function of sucrose concentration. From that he inferred that the electron density distribution was uniform within the particle and that any experimental scattering curve could be considered as the scattering shape function. Our experiments, as well as previous neutron data (Serdyuk, 1975; Stuhmann *et al.*, 1976; Serdyuk and Grenader, 1977) are at variance with this result. We observed a variation of the radius of gyration as a function of the contrast, in agreement with neutron results. However, because of the high average electron density contrast between particle and water, the curve recorded in the absence of sucrose yields a sufficient approximation of the scattering shape function out to $s = 0.016 \text{ Å}^{-1}$.

The determination of the scattering shape function of a model is a two-stage process, the digitalisation of the volume preceeding the computation proper. One starts with either a volume or a set of atomic coordinates. In the latter case, the solvent excluded volume has first to be determined. This is a difficult problem and only approximate methods could be

developed (Ninio *et al.*, 1972; Fedorov *et al.*, 1974). Once the volume is known, the scattering elements used for its description can be spheres so as to eliminate the spherical averaging step (Tolbert, 1971; Glatter, 1980) or cubes which fill space with no need for interpenetrating elements as is the case for spheres (Ninio *et al.*, 1972; Fedorov *et al.*, 1974).

Most of the methods used to compute scattering functions rely, in one way or another, on Debye's formula (Debye, 1915; Guinier and Fournet, 1955). Intensities are generally calculated in the whole 3-D reciprocal space before being spherically averaged. It is also possible to operate in real space, first calculating the distribution function of distances. This function can be obtained by histogramming the distances between scattering elements (Tolbert, 1971; Glatter, 1980) or the histogram can be calculated from the distances between randomly selected elements using, for example, a Monte-Carlo procedure (Pickover and Engelman, in press). In a different approach, Labichinski and Bradaczek (1977) developed a method in which the computation is performed using fast Fourier transform algorithm. All these methods are fairly flexible as regards resolution and precision of both the description of the model and the computation. Finally, these methods are not limited to the calculation of scattering shape functions and can accommodate different electron density levels.

Our approach operates in real space and makes use of cubic elements. We histogram the common volume to the particle and its translated ghost instead of distances, as explained above. One advantage of this procedure, otherwise equivalent to most other methods, is to allow a selection of the translations corresponding to any given value(s) of r and thus to restrict the computation of the auto-correlation function to those distances. With other procedures, this computation is an "all or nothing" process. In addition, with our method, the number of translations used in the spherical averaging procedure can be varied at will for each value of r , depending on the precision required. It is also easily programmed.

We have shown that the scale given by the above authors for their models of the 50S ribosomal subunit was either slightly higher than (15%), or consistent with, the scale derived from the volume determined experimentally, which is likely to be more accurate than values measured from electron micrographs. Furthermore, the values obtained for the radius of gyration after scaling are practically identical for the two models and very close to experimental value. Nevertheless, the differences in shape between the two models are mirrored by significant differences in the positions of the minima and the maxima of the scattering curve, even at low resolution (see Figure 4). In simple terms, it looks as if model II were too spherical: indeed, the positions of the two peaks on the curve of model II correspond to the first two subsidiary maxima of a sphere 200 Å in diameter close to the spherical envelope of model II. By contrast, model I shows significant departures from sphericity.

We were also interested in determining the sensitivity of the fit to the presence of some characteristic features of the model. We therefore computed the shape function of model I, leaving out most of the long lateral appendage. The curve obtained is similar in shape to the curve of model I but intensities are constantly lower in the angular range of interest: the R factor increases from 12% to 18%. Therefore, the method seems quite sensitive, given the low resolution of the present

work and the fact that the part of the appendage we left out only accounts for ~3% of the scattering mass. Finally, we stress that we have not taken experimental errors into account. Although we are aware that a rigorous treatment of errors is a prerequisite of this type of analysis, in the present case the differences between the two calculated curves are such that errors are negligible in comparison.

Our results demonstrate the potential of SXRS for a quantitative model analysis; clearly, this approach can be applied to a variety of biological structures and is not limited to data from electron microscopy. As regards X-ray scattering experiments, we may expect further improvements in the quality and resolution of the data due to continuous technological progress and particularly to the use of synchrotron radiation sources. For ribosomes, the next step in model calculations is the introduction of internal electron density fluctuations. The distribution of the different chemical components within a particle could be approached using information provided, for instance, by immuno-electron microscopy (Strycharz *et al.*, 1978; Dabbs *et al.*, 1981) or triangulation experiments with neutron scattering (Ramakrishnan *et al.*, 1981). Finally, more sophisticated data processing methods including a treatment of experimental errors are presently emerging (Moore, 1980; Taupin and Luzzati, in press) which should shortly open the way to further model refinement.

Materials and methods

50S subunit preparation

50S Ribosomal subunits were obtained from *E. coli* strain MRE600 (Dondon *et al.*, 1974). Briefly, ribosomes were washed in high salt 1 M NH_4Cl , then centrifuged in a 5 mM Mg^{2+} buffer in a Beckman Ti 15 zonal rotor at 22K r.p.m. for 15 h. This yields an homogeneous population of 70S Å ribosomes (Wishnia *et al.*, 1975; Debey *et al.*, 1975), also called tight couples (Hapke and Noll, 1976). The 50S ribosomal subunits were prepared by dissociation of the purified 70S ribosomes and separated by zonal centrifugation in a 10 mM Mg^{2+} , 400 mM NaCl buffer. Purified subunits were stored at -80°C in the following buffer (buffer B1): 10 mM Tris-HCl, pH 7.5, 8 mM $\text{Mg}(\text{CH}_3\text{CO}_2)_2$, 100 mM NH_4Cl , 1 mM DTT. All the scattering experiments were performed in buffer B1. Concentrations were determined by u.v. absorption on a Cary 118c spectrophotometer using the value $E_{1\%}^1\text{cm} = 145$ at 260 nm for the extinction coefficient (Hill *et al.*, 1969b).

SXRS experiments

Low-angle scattering experiments to yield the radius of gyration and intensity at the origin were performed with an Elliott rotating copper anode generator with microfocus setting. The experimental procedures are described elsewhere (Sardet *et al.*, 1976). The scattering angle is defined by $s = 2\sin\theta/\lambda$. The angular range recorded was 0.001 Å^{-1} to 0.02 Å^{-1} . Contrast variation experiments at higher angles were performed at the synchrotron radiation laboratory L.U.R.E. The camera has been described elsewhere (Koch *et al.*, 1981; Moody *et al.*, 1980). The angular range recorded was 0.0025 Å^{-1} to 0.05 Å^{-1} . In both cases the scattered intensity was compared with the energy of the incident beam and the experimental curves were normalised and put on an absolute scale (Luzzati, 1960; Sardet *et al.*, 1976). Collimation corrections were done according to Lake's procedure (1967). The intensity at the origin was determined by extrapolation of the Guinier plots (Guinier and Fournet, 1955).

Sucrose was used to increase the electron density of the solvent. 50S ribosomal subunit solutions were made by mixing stock solutions prepared in buffer B1 and buffer B1 containing 66% sucrose w/w. Solutions were made 1 mM DTT immediately prior to experiments. Concentrations were in the range 4–15 mg/ml for low-angle experiments (no interparticle interference effects were detectable in these conditions) and ~50 mg/ml for the high-angle experiments (interparticle interference effects at this concentration appeared to be negligible for $s \geq 0.007\text{ Å}^{-1}$). Fifteen curves were recorded for the low-angle region; typical exposure time was 10 h. Eleven curves were recorded at the L.U.R.E. for the high-angle region; typical exposure time was 20 min. All experiments were performed at $10^\circ\text{C} \pm 0.2^\circ\text{C}$.

Model measurements

Measurements of model I were made on rhodorsil (Rhône-Poulenc) moulds of the upper and lower halves of a plaster copy. The z coordinates were

measured on a square grid of 5 mm side (maximum dimension of the model: 11.2 cm). For model II, the x , y and z coordinates of ~400 points on the surface of a plaster copy were measured with the Machine de Mesure Tridimensionnelle (SEIV). Some points were interpolated in order to end up with the z_1 and z_2 coordinates of the surface on a grid of 4 mm side (maximum dimension of the model: 10.5 cm). The final accuracy of the z_1 and z_2 coordinates was $\pm 1\text{ mm}$ due to necessary mechanical manipulations of the models.

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