A NEW APPROACH FOR USING LABELS IN SMALL-ANGLE SCATTERING EXPERIMENTS

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1. Introduction

The molecular information which can be obtained from small-angle scattering (SAS) experiments in comparison to crystallography is drastically reduced for two main reasons.

(i) The random rotational motions of the molecules in solution eleminate direct access to the orientational information of the structure [1].

(ii) In SAS experiments there is no amplification of the scattered intensity by a lattice factor as in the case of crystals, therefore resolution is very low.

Due to these limitations a unique solution of a structure can normally not be given on the basis of SAS experiments alone.

The introduction of parameters characterizing the macromolecule was therefore of great advantage in the development of SAS [2–6]. The radius of gyration introduced in [2] is a famous example. Contrast variation was another successful method widely used in neutron small-angle scattering experiments [7,8]. By this procedure the scattering curve can be split up into so-called basic scattering functions which give useful information about different aspects of the structure. The triangulation method is another approach used for macromolecular assemblies, which can be reconstituted from their subunits. The relative positions of the various units is found by determining the distances between pairs of subunits synthetically unhanced in their scattering power [9–11]. Comprehensive reviews on SAS have appeared in [12–14].

This paper demonstrates a simple procedure by which additional information can be obtained from the small-angle scattering curve of a molecule which contains a small label of enhanced scattering power. Under these conditions the distance distribution function between the label and each segment in the macromolecule can be determined, which gives a more direct view of the structure. Experimentally, this method has some advantage in combination with anomalous scattering.

2. The method

A dilute solution of monodisperse scattering particles P is considered, each containing a label L of large scattering power rigidly bound to a specific site; e.g., in the case of X-rays the label may be a small cluster of heavy atoms or for neutrons a group of deuterated segments. Debeye's law describes the scattering of a molecule in dilute solution by:

$$J(s) = \sum_i \sum_j f_i f_j \frac{\sin 2\pi |r_i - r_j| s}{2\pi |r_i - r_j| s} \qquad (1)$$

where f_i and f_j are the coherent scattering lengths for the ith and jth atoms and r_i and r_j are vectors indicating their positions relative to an arbitrary origin. $s = 2 \sin \theta / \lambda$, where θ is half the scattering angle and λ the wavelength of the radiation used. Applying this formula to a labelled particle three terms arise:

$$J(s) = J_P(s) + J_{PL}(s) + J_L(s) \qquad (2)$$

$J_P(s)$ represents the interferences within the unlabelled particle and $J_{PL}(s)$ the interferences between the particle and the bound label. This term can be written as:

$$J_{PL}(s) = 2 f_L \sum_i f_i \frac{\sin 2\pi |r_L - r_i| s}{2\pi |r_L - r_i| s} \qquad (3)$$

where f_i and f_L are the scattering length of the ith segment in the particle and the label and r_i and r_L their positional vectors. $J_L(s)$ is the scattering contribution of the unbound label alone which is assumed to be small compared to the resolution achieved, so that this term is almost independent of s. The Fourier transform of $J(s)$ gives the small-angle correlation function or characteristic $H(r)$ [1,15], which is connected with the distance distribution $D(r)$ in the structure by:

$$D(r) = 4\pi r^2 H(r) \tag{4}$$

This distribution accounts for all distances between pairs of segments in the molecule. Applying the transform to $J_{\mathrm{PL}}(s)$, eq. (3), the distribution of distances between the label and every position in the structure is determined:

$$D_{\mathrm{PL}}(r) = 4\pi r^2 \int_0^\infty J_{\mathrm{PL}}(s) \frac{\sin 2\pi rs}{2\pi rs} 4\pi s^2 \, \mathrm{d}s \tag{5}$$

This is an interesting result, since having derived $J_{\mathrm{PL}}(s)$ experimentally $D_{\mathrm{PL}}(r)$ is simply obtained by Fourier transformation. $D_{\mathrm{PL}}(r)$ is a histogram of the distances between the segments in the molecule and the label. It provides a clearer picture of the structure since all distances are counted only with respect to one point, whereas $D(r)$ is much more complicated, since it gives the distances of all points with respect to all other points in the structure. For illustration three examples are given in the following:

(i) The particle P consists only of one atom i having a fixed distance $r_{i\mathrm{L}}$ with respect to the label. Then $D_{\mathrm{PL}}(r)$ is:

$$D_{\mathrm{PL}}(r) = 2f_{\mathrm{L}}f_i\,\delta(r - r_{i\mathrm{L}})$$

and has only one peak at $r = r_{i\mathrm{L}}$

(ii) The particle has a constant scattering length density. The value of $D_{\mathrm{PL}}(r)$ at position r is then directly proportional to the cross-sectional area cut out of P by a sphere with radius r around the position of the label. In this case $D_{\mathrm{PL}}(r)$ can provide a direct picture of the variations in the thickness of the particle.

(iii) The label is bound to one end of an elongated particle (e.g., TMV), then $D_{\mathrm{PL}}(r)$ is proportional to the profile of this particle along its major axis.

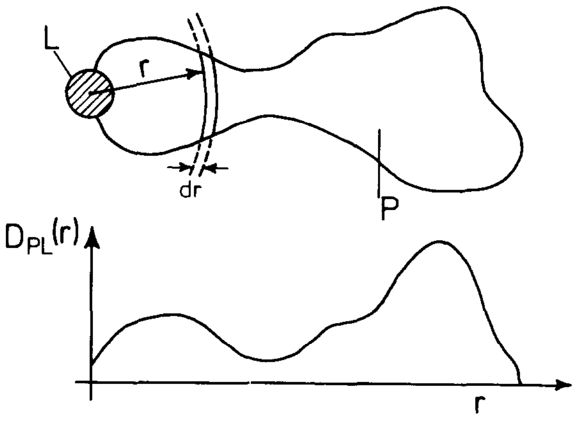

Fig.1. A schematic illustration of the method. In the upper part of the figure the label L bound to the particle P is shown. In the lower graph the corresponding distance distribution function $D_{\mathrm{PL}}(r)$ is seen, which shows at each value of r the sum of the scattering lengths of all atoms with that distance from the position of L.

3. Experimental determination of $J_{\mathrm{PL}}(s)$

Different procedures can be used to measure $J_{\mathrm{PL}}(s)$:

1. The scattering curves of two solutions A and B are subtracted. A contains the labelled and B the unlabelled particles. In this case $J_{\mathrm{L}}(s)$ in eq. (2) is calculated and separately subtracted from A.
2. Solution B contains the unlabelled molecule and the unbound randomly dispersed label. From the difference of the scattering curves of solution A and B, $J_{\mathrm{L}}(s)$ is now automatically subtracted.
3. The label consists of atoms which can be used for anomalous scattering, e.g., several iron atoms in the case of X-rays. In this case f_{L}, the form factor of the label, is a complex number which is strongly dependent on the wavelength λ [16]:

$$f_{\mathrm{L}} = f_{\mathrm{o}} + f' + if'' \tag{6}$$

Here f_{o} in the case of X-rays is the normal scattering length, which in the forward scattering direction is $f_{\mathrm{o}} = Ze^2/(mc^2)$, where Z is the number of electrons and $e^2/(mc^2) = 2.818 \times 10^{-13}$ cm is the Thomson scattering length. f' and f'' show large variations with λ in their amplitudes near the absorption edge. Measurements are performed on the same sample at two different values, λ_1 and λ_2. The experiments at wavelength λ_1 (indicated by the index (1)) can be described by:

$$J^{(1)}(s) = J_{\mathrm{P}}(s) + |f_{\mathrm{L}}^{(1)}|^2 + 2(f_{\mathrm{o}} + f'^{(1)}) \sum_i f_i \frac{\sin 2\pi|r_{\mathrm{L}} - r_i|s}{2\pi|r_{\mathrm{L}} - r_i|s} \qquad (7)$$

The second experiment with λ_2 will give an equivalent expression. After subtraction the following formula arises:

$$\Delta J(s) = \Delta(|f_{\mathrm{L}}|^2) + 2\Delta f' \sum_i f_i \frac{\sin 2\pi|r_{\mathrm{L}} - r_i|s}{2\pi|r_{\mathrm{L}} - r_i|s} \qquad (8)$$

where $\Delta J(s) = J^{(1)}(s) - J^{(2)}(s)$ etc.

Fourier transformation of $\Delta J(s)$ gives:

$$\int_0^\infty \Delta J(s) \frac{\sin 2\pi rs}{2\pi rs} 4\pi s^2 \, ds =$$

$$\Delta(|f_{\mathrm{L}}|^2)\delta(r) + 2\Delta f' \, h_{\mathrm{PL}}(r) \qquad (9)$$

where $h_{\mathrm{PL}}(r) = H_{\mathrm{PL}}(r)/(2\Delta f')$

The first term is easily separated because it is a strong δ-peak at the origin and $D_{\mathrm{PL}}(r)$ is determined from the rest. Thus by using the advantages of anomalous scattering $D_{\mathrm{PL}}(r)$ can be obtained with higher precision from only one sample.

4. Applications and outlook

The examples (ii) and (iii) of section 2 already show the essential applications of this method. For investigating the shape of a macromolecule in more detail it is necessary to study different samples each of which is labelled at another site.

If the same particle is labelled at different sites the distances of these labels to one label L could be seen in the $D_{\mathrm{PL}}(r)$ graph. In the next step the distances of these labels with respect another label are determined. Thus the triangulation procedure could be used more economically.

It should be noted, however, that the method discussed so far considers only small labels, so that the scattering of the label alone is almost independent of s in the range where the scattering is observed. In cases where this approximation can not be applied, interferences between all volume elements of the label and the particle have to be considered, which results in a complicated cross-correlation function for $H_{\mathrm{PL}}(r)$. How useful information can be obtained in these cases, especially when the label can be described by a spherical scattering length density, is discussed elsewhere.

Since heavy atom compounds are widely used in electron microscopy, several labels of different size and scattering power are available for X-ray studies. For determining $D_{\mathrm{PL}}(r)$ of small particles the following labels can be used, which carry in a cluster of about 4 Å in diameter, 4 Hg atoms, e.g., tetrakisacetoxymercurimethane [17], tetraacetoxymercurithiophene [18] and mercurated *N*-pyrroloisomaleimide [19]. Hexaphenylene mercury with 6 Hg atoms within a sphere of about 5.5 Å in diameter is another compound which could be chemically modified for specific binding [20]. For larger structures undecagold is a suitable label having 11 Au atoms in a cluster of 8 Å diameter [21]. Finally, a label of colloidal gold (diameter about 20 Å) coated with a monolayer of a specific macromolecule could be attached to very large particles [22].

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