On Soulé–Porod Plots of Protein X-Ray Scattering Data

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It is shown that inhomogeneous electron density distribution as represented either by a spherical model containing smaller spheres or by a sphere with radially periodic density does not produce positive slope in Soulé–Porod plots \( (Ih^4 \text{ vs } h^4) \) of protein small-angle scattering data. It is found that the introduction of small particles into the scattering population can produce the zero or positive slope commonly seen. It is also shown that intersubunit interference is able to account for the low 2\( \theta \) minimum or dip often observed in \( Ih^4 \text{ vs } h^4 \) plots of the scattering from multisubunit proteins.

Introduction

It is generally assumed that Porod’s law

\[
I(h)_{\text{asympt.}} = (\rho - \rho_0)^2 \frac{2\pi S}{h^4},
\]

where \( I(h)_{\text{asympt.}} \) is the scattered intensity in electron units for large \( h \), \( \rho \) is the average electron density in the scattering particles, \( \rho_0 \) is the electron density of the continuous medium, \( S \) is the total surface area of the particle, and \( h = 4\pi \sin \theta /\lambda \), is valid for any shape of the particle, so long as the orientations are random and so long as none of the particle dimensions are very small \( (hL \gg 1 \text{ for every dimension } L) \). This law is assumed to be valid for a mixture of dissimilar particles even with interparticle interference (Guinier, 1963). Using protein scattering data, Luzzati, Witz & Nicolaieff (1961a) have published experimental \( Ih^4 \text{ vs } h^4 \) curves, or Soulé–Porod plots, that agree with the plot expected from Porod’s law behavior. But many people (Luzzati, Witz & Nicolaieff, 1961b; Witz, Timasheff & Luzzati, 1964; Pessen, Kumasinski & Timasheff, 1971) have reported significant deviations from Porod’s law in Soulé–Porod plots of protein data. Various attempts have been made to explain these deviations (Luzzati, Witz & Nicolaieff, 1961b; Ruland, 1971). Luzzati et al. (1961b) attributed positive slope at higher \( \sin \theta \) in Soulé–Porod plots (Fig. 4a, for example) to inhomogeneous electron density within the macromolecule. This positive end-slope, which was proportional to concentration, was subtracted from the observed curve \( I(h) \), and the residual curve \( I^*(h) \) was used for further evaluation, as in (2):

\[
\lim_{h \to \infty} h^4 I(h) = A + \delta^* h^4
\]

\[
I^*(h) = I(h) - \delta^*.
\]

\( I^*(h) \) obeys Porod’s law, with \( \delta^* \) reflecting the internal structure of the particle. We will suggest that these deviations may not be entirely due to inhomogeneity in the particle, and we will show that introducing hypothetical internal structure into a spherical particle has very little influence on the Soulé–Porod plot in the \( 2\theta \) range commonly studied. Other possible explanations for the observed deviations from ideal Soulé–Porod behavior are offered. It is shown that the addition of smaller particles to the solution can lead to positive deviations in Soulé–Porod plots, and that subunit interference in multi-subunit proteins can account for the low \( 2\theta \) minimum often observed in the plots. Our qualitative treatment ignores any contributions from imperfect data handling such as incomplete collimation corrections.

Computational methods

Computations were first carried out to study the effect on \( Ih^4 \text{ vs } h^4 \) plots of density fluctuations (inhomogeneity) within the macromolecule. Here \( I(h) \) is the desmeared intensity, and Soulé–Porod plots are plotted as \( Ih^4 \text{ vs } h^4 \). Some workers have used smeared intensity \( J(h) \), in which case Soulé–Porod plots are plotted as \( Jh^3 \text{ vs } h^3 \) (Guinier & Fournet, 1955; Ruland, 1971).

A globular protein molecule was approximated by a sphere of 20 Å radius, which is approximately the size of an isolated monomer of L-asparaginase, a tetrameric enzyme being investigated in our laboratory. Internal structure of the particle was simulated by packing the spherical monomer with smaller spherical cells of uniform density and radius \( r \). The intercellular distance \( d_{ij} \) was made equal to \( 2r \). To calculate the coordinates of the cells, the molecule was divided into a hexagonal close-packed lattice with scattering cells placed at each lattice point (Kittel, 1968).

The calculation of the scattering from the molecule was based on the Debye equation:

\[
I(h) = \sum_{m=1}^{N} f_m \sum_{n=1}^{N} \frac{\sin (hd_{ij})}{hd_{ij}}
\]

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where \( N \) is the total number of scattering cells in the molecule, and \( f_i \) and \( f_j \) are the scattering factors of any pair of cells. Here, the cells are identical and

\[
f_i = \phi_i V_i \varphi_i(hr),
\]

where \( \phi_i \) is the averaged electron density of the \( i \)th cell, \( V_i \) is the volume of the \( i \)th cell, and \( \varphi_i(hr) \) is its scattering amplitude given by

\[
\frac{\sin(hr) - hr \cos(hr)}{(hr)^3}.
\]

Another inhomogeneous model was considered in which the protein molecule was divided into concentric shells of different electron densities (Oster & Riley, 1952). A simple case is a sphere of radius \( R \) with an internal structure which is radially periodic. The electron density is proportional to \( G(r) \), where

\[
G(r) = \cos^2(\pi mr/R) \text{ for } R > r > 0
\]

\[
G(r) = 0 \text{ for } r > R,
\]

\( m \) being the number of concentric shells. The scattered intensity is given by \( I = F^2 \), where

\[
F = \frac{\int_0^\infty 4\pi r^2 G(r) \left[ \frac{\sin(hr)}{hr} \right] dr}{\int_0^\infty 4\pi r^2 G(r) dr}
\]

\[
= \frac{(2\pi m + hR)\varphi(2\pi m + hR) - (2\pi m - hR)\varphi(2\pi m - hR) + 2hR\varphi(hR)}{2hR \{1 + 6/(2\pi m)^2\}}
\]

and \( \varphi \) has the form of equation (4).*

### Results and discussion

Fig. 1(a) shows the Soule–Porod (\( I h^4 \) vs \( h^4 \)) plots for the model made of small spherical cells packed into a single spherical molecule. These plots show that in the rather low \( h \) region where Porod's law has been

It should be pointed out that in Oster & Riley (1952), the denominator in (5) was evaluated incorrectly as just \( 2hR \).

minima at higher \( h \) are shown for spheres in Fig. 2. Interestingly, the minima for one of our spherical models with internal structure are smoothed at large \( h \) values (\( \sim 1.0 \)) giving rise to Porod's law behavior. This suggests that even at large \( hr \) values (\( \sim 200 \)), a plateau in \( I h^4 \) vs \( h^4 \) plots can arise from factors other than polydispersity in the sample. According to Ruland (1971), a finite width of density transition between the phases should produce negative deviations from

![Fig. 1. Calculated Soule–Porod plot for a 20 Å spherical particle (a) containing smaller spherical cells of radius \( r \), (b) with radially periodic electron density (\( m = \) number of concentric shells). In this and the following figures, 3° 20 (Cu K\( \alpha \) radiation) corresponds to an \( h^4 \times 10^4 \) of 208 Å\(^{-4}\).](image-url)
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Soulé–Porod behavior. However, we suggest that since our models (Fig. 1) and even homogeneous spheres (Fig. 5a) produce a negative slope in the Soulé–Porod plot, it may not always be correct to apply Ruland’s models directly to limited protein scattering data in support of presumed hydration shells on the surface of globular protein molecules.

Porod’s approximation (1) can be derived for a polydisperse solution of spherical particles with radii ranging from \(a_1\) to \(a_2\), if \((2a_1s - 2a_2s) \approx 1\) with \(s = h/2\pi\) (Guinier, 1963). For the low \(h\) range over which Porod’s approximation is commonly applied in protein studies one finds that \((a_1 - a_2) \approx 15\) Å. This molecular size difference is quite unrealistic for monodisperse globular proteins, which are characteristically of uniform shape and size. This might be another reason why one is often not able to observe a plateau region in \(Ih^4\) vs \(h^4\) plots with purified protein solutions. Therefore, an attempt was made to simulate the scattering curve observed experimentally by including in the calculation smaller particles along with protein molecules. A radius of 5 Å for the small particles was arbitrarily chosen and the radius of the protein molecule was kept at 20 Å as before. As the population ratio of the small particle to protein was increased from 0.4 to 3.7 it was observed that the Soulé–Porod plot changed its shape from that for a homogeneous sphere (with negative deviation) to that predicted by Porod’s law and observed for lysozyme in our laboratory and also by Luzzati et al. (1961a). The results are shown in Fig. 3. Although the range of sizes necessary to make Porod’s law rigorous is large, in practice much less polydispersity, coupled with imperfect collimation and imperfect collimation correction routines, is required. Thus, observed data from most protein systems will not produce such large negative deviations shown in Fig. 3(a). This calculation clearly indicates, however, that the observed scattering is strongly influenced by the presence in the protein solution of small particles such as peptide fragments or contaminants. The scattering from water is almost constant over this range. Thus, incomplete subtraction of water scattering would add an \(h^4\) term to that from the protein, giving positive slope to the Soulé–Porod plot.

We also wished to study the low 20 differences observed (Murthy & Knox, 1976) in \(Ih^4\) vs \(h^4\) plots of the monomeric and tetrameric forms of L-asparaginase (Fig. 4). Computations were done with a spherical homogeneous sphere to represent the monomer, and with a tetrahedral arrangement of these spheres to represent the tetramer. Similar calculations, but with a much larger number of subunits (60), have been pub-

![Fig. 2. Calculated Soulé–Porod plots at large \(h\) values for (a) a homogeneous sphere of 20 Å radius, (b) a 20 Å radius sphere made of 2.5 Å spherical cells. A sphere with radially periodic electron density gives a plot similar to (a).](image)

![Fig. 3. Three calculated Soulé–Porod plots for a mixture of spherical particles (\(x = \text{ratio of 5 Å particles to 20 Å particles}\)) and an experimental plot of Luzzati (1961a). \(s^2 J(s)\) vs \(s^2\) and \(k^4(h)\) vs \(h^4\) are equivalent (Guinier & Fournet, 1955).](image)
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Fig. 4. Experimentally observed Soule–Porod plot for (a) monomeric and (b) tetrameric form of E. coli L-asparaginase (Murthy & Knox, 1976).

Fig. 5. Calculated Soule–Porod plots for (a) a homogeneous sphere of 20 Å radius, and (b) an aggregate of four 20 Å spheres, placed at the corners of a tetrahedron of side 37 Å.

lished by Glatter (1972). Results obtained by the use of (3) and (4) are shown in Fig. 5. Though we could not simulate the experimental scattering curves at high 2θ, the calculated curves below $h^4 = 4 \times 10^{-4}$ appear to mimic the large low-2θ differences between the monomer and the tetramer. The dip at $h^4 = 3 \times 10^{-4}$ in the $Ih^4$ vs $h^4$ plot has also been observed in multimeric proteins by other workers (Witz, Timasheff & Luzzati, 1964), but without explanation. Our calculations show that intersubunit interference in the multimer is one factor which can account for this dip at low 2θ. This conclusion is reinforced by the fact that the dip is not observed in the dissociated monomer of asparaginase (Fig. 4a) or in monomeric lysozyme (Pessen, Kumosinski & Timasheff, 1971; Murthy & Knox, unpublished).

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References