On the Estimation of the Radius of Gyration of the Subunits of Macromolecular Aggregates of Biological Origin in situ

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Abstract

Radius of gyration estimates obtained for the subunits of macromolecular aggregates in situ by neutron scattering, with contrast matching techniques, are liable to serious systematic errors. An appropriate theory for these measurements is presented and its practical implications are explored.

Introduction

Many biological structures are well-defined assemblies of macromolecular subunits whose size and shape in situ one would often like to know. Recently, information of this kind has been sought by neutron small-angle scattering. A typical experiment involves the measurement of the scattering curve of an assembly which contains a subunit of distinctive scattering-length density, $Q$, in a solvent which contrast matches the remainder of the structure. The distinction between the subunit of interest and the rest of the assembly can be produced artificially by deuterium labelling (e.g. Stockel, May, Strell, Cejka, Hoppe, Heumann, Zillig, Crespi, Katz & Ibel, 1979) or be one based on naturally occurring differences in $Q$ between macromolecules of different chemical types (e.g. Stuhrmann, Haas, Ibel, DeWolf, Koch, Parfait & Crichton, 1976). The purpose of this paper is to point out the difficulties in interpreting 'subunit' scattering curves of this kind in terms of the structure of the subunits in question. The radii of gyration deduced from such data are liable to significant systematic errors.

Theory

Suppose an aggregate has been prepared with a single subunit deuterium labelled. The labelling creates a region of increased $Q$ superimposed on the normal scattering-length density distribution for the whole structure (including the subunit of interest in unlabelled form). What radius of gyration will be observed for this labelled structure? The labelled region will be characterized by a scattering-length density, $\delta Q$, which is the increment in average scattering-length density in the subunit of interest produced by labelling. The volume of the region of increased scattering-length density will be $V_s$, which is the volume of the subunit for all practical purposes. Let $R_d$ be the radius of gyration of the incremental scattering-length density distribution within $V_s$.

$$R_d^2 = \delta Q \int_{V_s} |r|^2 dV / \delta Q V_s = \int_{V_s} |r|^2 dV / V_s ,$$

(1)

where the integrals are volume integrals running over the labelled region. The vector $r$ is measured relative to the center of incremental scattering-length density, i.e. the point where

$$\delta Q \int_{V_s} r dV = 0 .$$

Typically, in the case being considered, $\delta Q$ would be given by

$$\delta Q = (b_o - b_H) ND/V_s ,$$

(3)

where $b_o$ and $b_H$ are the scattering lengths of deuterium and hydrogen respectively and $N_D$ is the number of positions within the labelled subunit substituted with deuterium non-exchangeably. $R_d$ will be close to the radius of gyration of the unlabelled subunit, in situ, and by measuring it a useful piece of evidence about the size and shape of the subunit will be obtained.

Let $R$ be the radius of gyration of the unlabelled structure, subunit and all.

$$R^2 = \int_{V_t} [\rho(r) - \rho_0] |r|^2 dV / \int_{V_t} [\rho(r) - \rho_0] dV ,$$

(4)

where $\rho_0$ is the scattering-length density of the solvent and $\rho(r)$ the scattering-length density of the unlabelled particle. The volume integrals run over the entire volume of the structure. As in (1), $r$ is measured relative to the center of scattering-length density in the particle, i.e. from the point where

$$\int_{V_t} [\rho(r) - \rho_0] r dV = 0 .$$

(5)

The labelled structure can be thought of as the superposition of the unlabelled scattering-length density distribution and the incremental scattering-length density distribution, which to first approximation is constant over the labelled region. Then, following the usual law for the addition of radii of gyration, the radius observed for the labelled structure, $R_{obs}$, will be
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\( R_{\text{obs}}^2 = f_1 R_e^2 + f_2 R_i^2 + f_1 f_2 |d_{ul}|^2 \)  
(6)

(Damaschun, Fitchner, Purschel & Reich, 1968; Damaschun & Purschel, 1970). In (6), \( d_{ul} \) is the vector from the center of incremental scattering mass of the labelled subunit (equation 2) to the center of scattering mass of the unlabelled structure (equation 5). Let \( V_e \) be the volume of the entire structure and \( \Delta \rho \) the difference in average scattering-length density between the unlabelled structure and the solvent.

\[ \Delta \rho = \int_{V_t} \rho(r) dV/V_t - \bar{\rho} . \]  
(7)

Then, in (6),

\[ f_1 = \delta \rho V_e / (\delta \rho V_i + \Delta \rho V_i) , \]
\[ f_2 = 1 - f_1 . \]  
(8)

If \( R_e \) were independent of \( \Delta \rho \), i.e. independent of \( \bar{\rho} \), then (6) would imply that at \( \Delta \rho = 0 \), \( R_{\text{obs}} = R_e \) as has often been assumed. However, \( R_e \) in general is not independent of \( \Delta \rho \); it has a quadratic dependence on \( \Delta \rho \):

\[ R_e^2 = R_c^2 + \alpha / \Delta \rho - \beta / \Delta \rho^2 . \]  
(9)

(Ibel & Stuhrmann, 1976; Cotton & Benoit, 1975; Luzzati, Tardieu, Mateu & Stuhrmann, 1976). In (9), \( R_c \) is the radius of gyration of a structure having the same shape and size as the whole aggregate, but no internal variations in scattering-length density:

\[ R_c^2 = \int_{V_t} |r|^2 dV/V_t . \]  
(10)

In (10) \( r \) is measured from the point where \( \int_{V_t} r dV = 0 . \)  
(11)

In both (10) and (11) the volume integrals run over the volume of the whole structure.

\[ \alpha = \int_{V_t} \bar{\rho}'(r) |r|^2 dV/V_t , \]  
(12)

where \( \bar{\rho}'(r) \) is the fluctuation of \( \rho(r) \) about its mean value, \( \bar{\rho} ; \)

\[ \bar{\rho} = \int_{V_t} \rho(r) dV/V_t . \]  
(13)

Let

\[ \gamma = \int_{V_t} \bar{\rho}'(r) r dV/V_t . \]  
(14)

Then

\[ |\gamma|^2 = \beta . \]  
(15)

\( d_{ul} \) is also dependent on \( \Delta \rho \). Let \( r_v \) be the vector from the center of incremental scattering mass of the labelled region (equation 2) to the center of the volume of the unlabelled structure (equation 11). With equation (12) from the work of Luzzatti et al. (1976), it is easy to show that

\[ \bar{d}_{ul} = r_v + \gamma / \Delta \rho . \]  
(16)

Substituting (8), (9), (15) and (16) into (6), one obtains an expression which can be rearranged to give the following:

\[ R_{\text{obs}}^2 = R_c^2 + \frac{1}{\Delta \rho'} \left[ \alpha + \frac{\delta \rho V_e}{V_i} (R_e^2 - R_i^2 + r_v^2) \right] \]
\[ - \frac{1}{\Delta \rho'^2} \left( \gamma - r_v \frac{\delta \rho V_e}{V_i} \right)^2 , \]  
(17)

where \( \Delta \rho' \) is the contrast between the solvent and the entire labelled structure:

\[ \Delta \rho' = (\delta \rho V_i + \Delta \rho V_i) / V_i . \]  
(18)

(Because the labelled region is contained within the unlabelled structure both \( R_e \) and \( V_e \) are unaffected by labelling.) At \( \Delta \rho = 0 , \)

\[ R_{\text{obs}}^2 = R_e^2 + \alpha / \bar{\rho} - \beta / \bar{\rho}^2 . \]  
(19)

Thus, because of the internal fluctuations in contrast in the unlabelled aggregate, the radius of gyration when the labelled structure is contrast matched is not that of the labelled region as an isolated structure. Nor, in general, can it be anticipated that there will be a contrast at which \( R_{\text{obs}} = R_e \).

Note that because \( \bar{\rho} \) is created by covalent replacement of H with D, it is independent of the isotopic composition of the solvent. Therefore, \( R_e \) depends on labelling only and will be a constant feature of the labelled structure.

Discussion

The theory elaborated above is appropriately applied to the study of aggregates where both unlabelled and labelled forms can be prepared. In this case, \( R_e, \alpha \) and \( \beta \) can in principle be evaluated experimentally from radius of gyration data collected as a function of \( \Delta \rho \) on the unlabelled structure. One could imagine using these values to correct the observed radius of gyration at contrast match (see equation 19) for its \( \alpha \) and \( \beta \) contributions. However, even in this most favorable case, \( r_v, \gamma \) would be unknown and an uncertainty remain in the estimate for \( R_e \) of the order of \[ \sqrt{2 |\gamma| / |\gamma| \bar{\rho}^2 \bar{\rho}^2} [\gamma(\rho V_i \bar{\rho} V_0)]^{1/2} \] , \( |\gamma| = \beta^{1/2} . \)

A variant of this experiment arises when the subunit in question differs naturally in contrast from the rest of the aggregate of which it is a part due to its chemical nature. The same theoretical treatment holds provided some changes in definitions are made. Firstly, \( R_e, \alpha \) and \( \beta \) in (9) must refer to the part of the structure whose scattering is to be suppressed by contrast matching. Secondly, \( \delta \rho \) must be replaced by \( \Delta \rho' \), the difference in average scattering-length density between the subunit of interest and the solvent. Thirdly, the volume of the
'labelled' subunit is not contained in $V_\gamma$. A more complicated form of (17) results, but at $\Delta Q = 0$ an expression similar to (19) is recovered again. In this case $R_d^2$ will be the radius of gyration of the subunit of interest at the contrast in question, and there will be an additional correction term of the form

$$[-2V_\gamma V_\gamma \cdot \gamma_r / (V_\delta Q)^2]$$

where $\delta Q$ is now the contrast between solvent and the subunit in question. In this expression $\gamma_r$ is $\gamma$ (equation 14) evaluated for the subunit of interest and $\gamma_r$ is the corresponding quantity for the parts of the structure being contrast matched. From an experimental standpoint, this situation is difficult to deal with. Both $\alpha$ and $\beta$ refer to the scattering of the 'unlabelled' part of the complex as it exists in situ. Scattering studies on that portion of the structure free in solution, while helpful in estimating $\alpha$ and $\beta$, would not yield the precise values needed.

Quantitatively, what are the implications of this theory? Suppose an attempt is made to measure $R_d$ for a 20 000 dalton protein subunit in a nucleoprotein like the 50S ribosomal subunit of *E. coli* ($M_r = 1.5 \times 10^6$). Suppose, in addition, that the internal variations in $\rho$ in that structure have been suppressed by differential deuterium labelling of its protein and nucleic acid parts (Stuhrmann et al., 1976; Crichton, Engelman, Haas, Koch, Moore, Parfait & Stuhrmann, 1977). As a consequence, $\alpha$ is reduced to 0 as well as any uncertainties due to $\alpha$ and $\beta$ of 10$^{-15}$ of the 1H structure. Thus it will often be the case that $\beta$ contributions much too small to measure can upset determinations of subunit radii of gyration.

It is interesting that, in general, one anticipates that $\beta$ values for large macromolecules will often be small. Consider $R_{obs}^2$ for a two-subunit structure. Assign each a dependence of radius of gyration on $\Delta Q$ which follows (9) and assume both have the same average scattering-length densities. The vector joining their centers of scattering mass will be $[d_{12} + (r_1 - r_2)/\Delta Q]$. Substitution into (6) yields an expression of the form

$$R_{obs}^2 = \frac{V_1}{V_1 + V_2} (R_2^2 + \alpha_1/\Delta Q - \beta_1/\Delta Q^2)$$

$$+ \frac{V_2}{V_1 + V_2} (R_2^2 + \alpha_2/\Delta Q - \beta_2/\Delta Q^2)$$

$$+ \frac{V_1 V_2}{(V_1 + V_2)^2} [d_{12} + (r_1 - r_2)/\Delta Q]^2,$$

(21)

where $V_1$ and $V_2$ are the volumes of the two subunits. Minor algebraic manipulation will reveal that $\beta$ for the joint structure will be $(V_1 \gamma_1 + V_2 \gamma_2)^2/(V_1 + V_2)^2$. For an $N$ subunit (or folded domain) structure, $\beta_N$, the $\beta$ for the whole structure will be

$$\beta_N = \left[ \sum (V_i/\gamma_i) \right]^2,$$

(22)

where $V_1, V_2, \ldots, V_i$ are the volumes of the subunits and $V_\gamma$ the volume of the whole structure.

Assuming the subunits in an assembly are about the same size and have similar values for $\beta$, $\beta_i$, the $\beta_N$ can be no larger than $\sim \beta_i$. If the assembly has point-group symmetry, $\beta_N$ can be 0. If the subunits associate 'randomly', then $\beta_N \approx \beta_i/N$. Thus $\beta$ will tend to decrease with assembly size. On the basis of data on molecules of the appropriate size and type for the subunits in a structure of interest it might be possible to arrive at a plausible estimate for $\beta$ for a large assembly well below the limits of what could be verified experimentally.

Three conclusions can be drawn. Firstly, *in situ* radius of gyration estimates obtained by the methods outlined above are subject to serious qualifications, and many such estimates exist in the literature whose validity needs to be reassessed. These qualifications stem from the variations in $\rho$ within the parts of a structure which are contrast matched. All experiments of this kind should include estimates of the size of the
uncertainty expected from this source. Secondly, it seems a reasonable inference that if these variations affect the radius of gyration observed, they will also contribute to 'subunit' scatter at angles outside the Guinier region. Before interpretation of extended scattering curves of this kind is undertaken this problem deserves careful attention. Thirdly, (19) suggests an experimental strategy which should yield \( R_d \). \( R_{\text{obs}} \) should be measured on a set of samples containing the subunit of interest labelled to varying levels of \( \delta \)g in the same unlabelled background. \( R_{\text{obs}} \) plotted versus \( 1/\delta g \) extrapolated to \( 1/\delta g = 0 \) should give \( R_d \). One suspects that in systems where reliable data could be obtained, \( R_{\text{obs}} \) is likely to be close to \( R_d \) anyway, and that in those cases where such extrapolation is essential, it will be very hard to carry out in practice.

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References