

19. OTHER EXPERIMENTAL TECHNIQUES

19.4.4. Distance measurements

19.4.4.1. Theory and background

By measuring distances between chemically specified points in a macromolecule or macromolecular complex, useful information may be obtained concerning its structure. In the case of a complex with many subunits, successive inter-subunit distance measurements can be combined by triangulation to generate a three-dimensional map specifying the relative positions of subunits and, perhaps, their individual radii of gyration. The only uncertainty is the handedness if more than three distances are combined. A number of approaches exist for making distance measurements, including NMR, fluorescence and electron-microscope techniques in which labelling is employed. Neutron scattering provides a particularly simple approach to such measurements in a conceptual sense. If we consider two labelled centres in a large macromolecule or complex, the interference of the scattering from the two labelled positions, $I_x(Q)$, can be separated from the scattering of the rest of the complex by measuring the scattering of the unlabelled complex, $I(Q)$, the two singly labelled complexes, $I_1(Q)$ and $I_2(Q)$, and the doubly labelled complex, $I_{12}(Q)$, as they are related by

$$I_x(Q) = [I_{12}(Q) + I(Q)] - [I_1(Q) + I_2(Q)]. \quad (19.4.4.1)$$

Using the Debye relationship, it can be shown that the cross term will have the approximate form

$$I_x(Q) = 2f_1f_2 \sin(d_{12}Q)/d_{12}Q, \quad (19.4.4.2)$$

where the interference intensity results from the correlation between radiation scattered from sites 1 and 2, with scattering strengths f_1 and f_2 . It is a damped sinusoidal fringe with a periodicity reciprocally related to the separation between the scattering regions. If the scattering regions are very small compared with the object, there will be nodes at equal intervals in Q ; if they are not, $I_x(Q)$ will be a sum of all cross correlations of label positions in the two regions.

19.4.4.2. Neutron distance measurements

In the neutron case, the usual application involves deuterium labelling of positions in the two regions, and the cross term becomes

$$I_x(Q) = 2(b_D - b_H)^2 \sum_i \sum_j (\sin d_{ij}Q)/d_{ij}Q, \quad (19.4.4.3)$$

where the first sum runs over all labelled positions in region 1 and the second over all labelled positions in region 2.

The interference experiment as a technique for studying macromolecules in solution was first proposed in 1947 as an X-ray scattering measurement in which the labels are heavy atoms (Kratky & Worthmann, 1947). It has been discussed several times since and tested (Hoppe, 1972; Vainshtein *et al.*, 1970); however, no biological application has ever been published, since the signals are small (Hoppe, 1972). In 1972, the interference idea was discovered again, but in the context of neutron scattering (Engelman & Moore, 1972). Initially, it was thought that the distance measurement would follow from inspection of the damped sinusoidal term, but it soon became evident that the size and shape of the labelled regions would have an important influence. Using a power series expansion like that originally employed by Guinier, the previous expression becomes (for small Q)

$$I_x(Q) \propto [1 - (1/6)(R_i^2 + R_j^2 + d_{ij}^2)Q^2],$$

where R_i and R_j are the radii of gyration of the subunits *in situ*, and d_{ij} is the separation of their centres of mass. It follows that (Moore *et al.*, 1978; Stöckel *et al.*, 1979)

$$R_g^2 = (1/2)(R_i^2 + R_j^2 + d_{ij}^2). \quad (19.4.4.4)$$

The second moment of a length distribution, M_{ij} , can be related to a radius of gyration. If $p_{ij}(r)$ is the length distribution of the cross term $I_x(Q)$, then

$$M_{ij} = \int r^2 p_{ij}(r) dr = 2R_g^2.$$

Hence (Moore *et al.*, 1978)

$$M_{ij} = R_i^2 + R_j^2 + d_{ij}^2. \quad (19.4.4.5)$$

Thus, M_{ij} is the parameter that contains the information in the difference experiment. If the radii of gyration of the labelled regions are small, then the distance is well measured in a single experiment. This would be the case, for example, if the labels were single heavy atoms in an X-ray experiment. However, in most complexes of macromolecules there will be many pairwise protein-protein relationships where the radii and the separation of centres have comparable magnitudes. One approach to extracting distance information is to know the radii of the subunits *in situ*, by estimation from their molecular weights, by measurement of the isolated subunits or from triple isotopic substitution (see above). The first approach is the least desirable but the easiest, and the last approach is correct but laborious.

If a complex has eight or more distinct subunits, the number of possible measurements of M_{ij} , $n(n-1)/2$, is sufficient to solve for the distances and radii, enriching the information obtained from the experiment. The minimum number of measurements required is $4n-6$, and for large complexes, such as the ribosome, a great excess of possible measurements exists, permitting refinement of the information through redundancy. Moore & Weinstein (1979) have described analytical methods for solving the problem with correct error propagation.

19.4.4.3. The statistical labelling method

In many biological assemblies, multiple copies of a given subunit are found. In such cases, statistical labelling methods provide useful information. The basic concept is to produce two samples in which labelled subunits are randomly mixed with unlabelled subunits within the particles, and in which the ratios of labelled to unlabelled subunits is different. The difference in the scattering from the two samples will then contain shape and orientation information for the subunits (Fujiwara & Mendelson, 1996). A simple case is that of a dimer in a larger complex. Random introduction of equimolar labelled (D) and unlabelled (H) subunits will result in a mixture of complexes, where HH:HD:DH:DD = 1:1:1:1. A sample can be prepared with an equimolar mixture of all H and all D so that HH:DD = 1:1. The difference in scattering between these samples will be simply the interference cross term for the dimer, but at half the weight it would have in the case where the subunits were distinctly labelled.

19.4.5. Practical considerations

19.4.5.1. Feasibility

For a biological scientist, the first question is whether knowledge of the parameters that can be obtained from neutron scattering is of interest for the biological problem under consideration. If the answer is affirmative, the best course of action is to make contact with individuals who have conducted experiments in the past, as well as with biologists at a neutron-scattering facility. As a preliminary guide, a few general points are pertinent.

For a solution-scattering experiment on an unlabelled sample, typical sample volumes are 0.1–0.2 ml at concentrations of around