AN X-RAY METHOD FOR DETERMINING DISTANCES BETWEEN HEAVY ATOMS IN MACROMOLECULES IN SOLUTION AND ITS APPLICATION TO THE STUDY OF GRAMICIDIN C DERIVATIVES

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Abstract

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AN X-RAY METHOD FOR DETERMINING DISTANCES BETWEEN HEAVY ATOMS IN MACROMOLECULES IN SOLUTION AND ITS APPLICATION TO THE STUDY OF GRAMICIDIN C DERIVATIVES

As is known, heavy atoms make the greatest contribution to the amplitude of X-ray scattering. This is the basis, in particular, of the "heavy-atom method" in crystal X-ray diffraction. We shall show here that the possibility of determining the mutual arrangement of heavy atoms is retained—although in a somewhat more restricted form—also in the case of maximally disordered systems: solutions of macromolecules, where the diffraction pattern is poor and concentrated in the region of small angles. From the pattern of small-angle scattering one usually determines only the large-scale geometrical parameters of the macromolecule as a whole: radius of gyration, volume, magnitude of anisometry, etc. However, if the molecule contains several heavy atoms, then, in addition to the indicated characteristics, their mutual arrangement can also be determined. This may prove useful in studying the structure of dissolved protein and other biological molecules, in which certain functional groups can often be chemically "labeled" with heavy atoms.

Let us consider the case where two heavy atoms T_1, T_2, separated from one another by a distance r_0, have been added to the electron density of the molecule $\rho_M(\mathbf{r})$. It is essential that the total charge of all (light) atoms of the molecule $\int \rho_M \, dv$ considerably exceed the charge of the heavy atoms; however, ρ_M is an approximately uniform distribution with relatively low scattering power, whereas the electron density $\rho_T = \rho_{T_1} + \rho_{T_2}$ is large and concentrated in the small volume of these atoms. Then the scattering intensity by the molecule is written as the Fourier transform of the self-convolution of the electron density $\mathfrak{F}[\rho_M + \rho_T]^2$ (1):

$$I(s) = \mathfrak{F}[\rho_M^2] + \mathfrak{F}[\rho_T^2] + 2\mathfrak{F}[\rho_M \rho_T], \tag{1a}$$

and, for the case of a system of randomly arranged molecules under spherical averaging,

$$I(s) = K\left[N^2\Phi_M(s) + 2f_{T_1}f_{T_2}\left(1 + \frac{\sin sr_0}{sr_0}\right) + W\right], \qquad (1b)$$

where $\Phi_M(s) = \mathfrak{F}[\rho]^2$. In formula (1b), K is the number of molecules in solution; N is the number of electrons in the macromolecule; f_{T_1}, f_{T_2} are the atomic factors of the heavy atoms; r_0 is the distance between them; $s = (4\pi\sin\theta)/\lambda$. The third term $W = 2\mathfrak{F}[\rho_M\rho_T]$ reflects the interference between the molecule and the heavy atoms. We assume that intermolecular interference is absent and that scattering by the solvent has been eliminated. Note that expression (1b)

is easy to rewrite for the case when there are not two heavy atoms, but m, by replacing the second term by $\sum_i\sum_j f_i f_j(\sin sr_{ij})/sr_{ij}$ [2].

The first term (1a, b) describes the central, sharply decreasing maximum of small-angle scattering, which makes it possible to find the geometrical characteristics of the molecule noted above. The information of interest to us on $r_{T_1T_2}$ is contained in the second term, which, on the contrary, is a series of distinct maxima whose positions and intensities are unambiguously related to the distances between the heavy atoms. These maxima are exactly the same as, for example, in electron diffraction of gaseous diatomic molecules– "dumbbells."

Let us now estimate the ratio of the three components of expression (1b). Consider the following simple model: a sphere of radius R with uniform electron density (a macromolecule), at opposite ends of whose diameter two heavy atoms are located. In this case the scattering intensity is expressed as

$$I(s) = K\left[N^2\Phi^2(s) + 2f_T^2(s)\times\left(1 + \frac{\sin 2sR}{2sR}\right) + 4Nf_T(s)\Phi_s\frac{\sin sR}{sR}\right], \qquad (2)$$

Fig. 1. Normalized components of the scattering intensity by a homogeneous sphere of radius R with "point" scatterers attached along the diameter.
1–$9(\sin sR - sR\cos sR)^2/(sR)^6$;
2–$[1 + (\sin 2sR)/2sR]/2$;
3–$[3(\sin sR - sR\cos sR)/(sR)^3][\sin sR/sR]$

$$\Phi(s) = 3(\sin sR - sR\cos sR)/(sR)^3$$

is the scattering amplitude for a sphere of radius R, normalized to $\Phi(0) = 1$ [2]. In Fig. 1 all three terms of (2) are presented, divided respectively by N^2, $4f_T^2(s)$, and $4Nf_T(s)$.

The intensity of the first term Φ^2 falls rapidly and, beginning with $sR = 7$, amounts to $10^{-3}\Phi(0)$. The magnitude of the third term also decreases quite

rapidly, although somewhat more slowly than Φ^2, oscillating about zero. The contribution from the two heavy atoms is a series of distinct maxima and minima slowly decreasing in amplitude and oscillating about the line $I = 1/2$. Therefore, beginning with a certain sR, the second term becomes predominant. As a criterion for the possibility of separating the contribution made to the diffraction pattern by the heavy atoms against the background of the central small-angle scattering, one may adopt the condition that the intensities of the first and second terms of equation (2) be equal. This gives $N^2 \cdot 10^{-3} = 2Z^2$, where Z is the number of electrons in the heavy atom, i.e. $N/Z \cong 50$. In this estimate $f_T(s)$ was taken equal to Z, since the decrease of $f_T(s)$ at small scattering angles may be neglected. In this case the intensity of the third term, $4 \cdot 50 \cdot Z^2/300 \cong Z^2$, is less than the contribution from the second term.

Thus, we see that there are grounds for obtaining a noticeable effect from the second term of expression (1) at the ratio $N/Z = 50$. In other words, for the heaviest atoms ($Z \cong 10^2$) this corresponds to a molecular weight of about 10^4.

For other models—non-spherical ones—the estimate will be even more favorable, since the shape maxima in this case become more diffuse and weak, and against their background it is easier to notice the "dumbbell effect," although the overall decrease of the central maximum is somewhat slowed.

Solvents in which macromolecules are usually studied reduce their scattering, almost without changing the scattering by the heavy atoms; this

allows our estimate to be shifted toward larger macromolecules. On the other hand, slit collimation (necessary in these experiments) slows the fall of the curve, which makes it difficult to isolate the maxima caused by the heavy atoms.

The experimental verification of the method was carried out for three substances. The first model compound was diiodoanthraquinone. The experiments were performed on a small-angle X-ray apparatus developed in the Protein Structure Laboratory of the Institute of Crystallography, Academy of Sciences of the USSR ([3]). The scattering curve of a solution of diiodoanthraquinone in sulfuric acid, representing the difference between the scattering of the solution of the substance under study

[Fig. 2 and Fig. 3]

Fig. 2. Scattering intensity by diiodoanthraquinone
Fig. 3. Scattering intensity by derivatives of gramicidin with heavy atoms. 1 —iodogramicidin, 2 —mercurygramicidin

and the pure solvent, is shown in Fig. 2. The maximum of interest to us is clearly visible. (Cu K_α radiation was used throughout.)

The distance between the heavy atoms, calculated from the position of the maximum, is 9.4 Å, which is close to the value of 9 Å known from data on the crystal structure of diiodoanthraquinone ([4]).

Next, more complex compounds were studied—derivatives of gramicidin C, whose geometry is largely unknown. The cyclic decapeptide gramicidin C (molecular weight 1213) has the following sequence of amino-acid residues:

(1)		(2)		(3)		(4)		(5)
valine	—	ornithine	—	leucine	—	phenylalanine	—	proline
|								|
proline	—	phenylalanine	—	leucine	—	ornithine	—	valine
(5′)		(4′)		(3′)		(2′)		(1′)

Two compounds were investigated: iodogramicidin hydrochloride, with iodine atoms attached to the phenylalanine residues (positions 4, 4′), and mercury-gramicidin hydrochloride, with mercury atoms attached to the ornithine residues (positions 2, 2′). Various models of the gramicidin molecule, for which no direct structure determination has yet been carried out, show that the distances between opposite sides of the gramicidin ring may be 8-15 Å.

The small-angle scattering curves for the gramicidin compounds are shown in Fig. 3 (here also the difference between the scattering by the solution and by the pure solvent is plotted). "Dumbbell-shaped" maxima are found on both curves. The distances found from them proved to be as follows: 9.7 Å for iodogramicidin and 10.4 Å for mercurygramicidin. The data obtained may assist in choosing a model of gramicidin, if this molecule is sufficiently stable and does not change its conformation upon attachment of heavy atoms.

In conclusion, let us dwell on some prospects of this method. One of them is the use of a difference technique: measurement of the scattering curves of solutions of molecules containing heavy atoms and of the "free" initial molecules. The difference of the intensities will give, in pure form (with the scattering by the solution already excluded), the last two terms of relations (1a, b):

$$\Delta I(s) = \mathfrak{F}[\rho_T^2] + 2\mathfrak{F}[\rho_M \rho_T]\,. \tag{3}$$

It is clear that here the maxima due to scattering by the heavy atoms will appear more distinctly, which will make it possible to determine r_0 for still heavier macromolecules, i.e., for proteins.

The use of such a difference technique will make it possible to obtain additional information also on the structure of the molecule itself, i.e., on $\rho_M(r)$. In the ordinary small-angle technique, the source of information on $\rho_M(r)$ is $\mathfrak{F}[\rho_M^2]$. If, however, we apply the difference technique described, then after determination of r_0 one can calculate $\mathfrak{F}[\rho_T^2]$ and subtract this term as well from (3), which will give $\mathfrak{F}[\rho_M \rho_T]$ (cf. Fig. 1). After inverse Fourier transformation of this term, the electron density of the molecule appears in a new form, and its geometrical parameters can be found quite independently. It is interesting to note that in

this case it is more convenient to introduce into the molecule not two (or more), but one heavy atom—then $\mathfrak{F}[\rho_T^2]$ will have no nonzero maxima at all.

Another possible way to improve the possibilities for detecting heavy atoms consists in artificially raising the electron density of the solvent to the average electron density of the molecule. Then subtraction of the scattering by the solvent will simultaneously be subtraction of the scattering by the molecule, and the difference will represent $\mathfrak{F}[\rho_T^2]$ in pure form.

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Note: Figure translations are in progress. See original paper for figures.