

Professor G N Ramachandran's Contributions to X-ray Crystallography

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K Venkatesan was on the faculty of the Department of Organic Chemistry, Indian Institute of Science from 1971-1992. After retirement he was the INSA senior scientist till 1997. His area of specialization is chemical crystallography with particular reference to structure-reactivity correlations in the crystalline state.

This article presents briefly the original contributions of Professor G N Ramachandran to the methods of the structure determination by X-ray diffraction of crystals.

Introduction

Crystals are composed of groups of atoms repeated at regular intervals, with the same orientation, in three-dimensions. To view atoms that are separated by distances of the order of 10^{-8} cms the appropriate radiation would be X-rays with the wavelength $\lambda \sim 10^{-8}$ cm $\sim 1\text{\AA}$. The scattering of X-rays by atoms in the crystal produces Bragg reflexions each with a different amplitude $|F(hkl)|$ which is called the structure amplitude and $\alpha(hkl)$ the relative phases. The expression for the complete wave scattered by the Bragg plane (hkl) is

$$F(hkl) = \sum_1^n f_n \exp 2\pi (hx_n + ky_n + lz_n), \quad (1)$$

where f_n is the scattering factor or the power of the n th atom to scatter X-rays. It depends upon the atomic number of the scattering atom. $F(hkl)$ is a complex quantity associated with it an amplitude $|F(hkl)|$ and phase angle $\alpha(hkl)$. In general the phase angle can take any value between θ and 2π . However, in a crystal with a centre of symmetry, α is limited to either 0 or π .

As the electron density distribution in a crystal is periodic in three dimensions, it can be expressed as a three-dimensional Fourier series

$$\rho(xyz) = \frac{1}{V} \sum_h \sum_k \sum_{l=-\infty}^{\infty} |F(hkl)| \cos [2\pi (hx + ky + lz) - \alpha(hkl)]. \quad (2)$$

The electron density $\rho(xyz)$ at position (xyz) in the unit cell of volume V , is expressed in electrons per cubic Å, and is maximum near atomic centres. The summation is to be taken over all the observed Bragg reflexions. Since the intensities can be measured, the structure amplitudes $|F(hkl)|$, can be obtained from a suitable experiment. However, to reconstruct the electron density distribution by adding the component waves we need to know not only their amplitude $|F(hkl)|$ but also the relative phases $\alpha(hkl)$, which are not experimentally available. It is therefore not possible to calculate directly $\alpha(xyz)$ in a straight forward way. This has been the fundamental problem of X-ray analysis until the so-called direct methods were developed after 1960. Four different approaches are available for tackling the phase problem. These are (a) the heavy atom method (b) isomorphous replacement method (c) anomalous dispersion method and (d) the direct method.

G N Ramachandran's important contributions have been concerned with the (a) the anomalous dispersion method for solving the phase problem and (b) determination of the crystal structures using Fourier syntheses of different kinds when only part of the structure is known.

Anomalous Dispersion Method

When the frequency of the incident X-rays (ω_i) is far from the natural absorption frequency of a scattering atom, the atomic scattering factor f_0 is real and positive. However when ω_i is close to and slightly larger than the characteristic absorption frequency of say K -electrons, then f becomes complex and can be written as

$$f = f_0 + \Delta f' + i\Delta f'' \quad (3)$$

Although the value of $\Delta f''$ tends to be much smaller than f_0 , the presence of the term $\Delta f''$ can lead to important effects. According to Friedel's law $|F(hkl)|^2 = |F(\bar{h}\bar{k}\bar{l})|^2$. But this law does not apply to a non-centrosymmetric crystal if there is an anoma-

Box 1

With normal light the phase problem is solved by using a suitable lens system. But for X-rays this process cannot be carried out by lenses since the refractive index of X-rays is virtually close to unity. So the recombination of the Bragg waves has to be achieved mathematically.

Box 2

The optical activity of a molecule is entirely due to the asymmetry (or the chirality) of the molecular structure. It was not known which of the two possible enantiomorphous structures i.e., the original molecule and its non-superposable mirror image, corresponded to the dextrorotatory isomer and which to the laevorotatory. Normally used X-ray wavelength cannot distinguish between them.

anomalous dispersion. The phase, $\alpha(hkl)$, associated with a reflexion (hkl) can be calculated from a knowledge of the difference of the intensities $I(\bar{h}\bar{k}\bar{l})$ and $I(hkl)$ and the position of the anomalously scattering atom.

If the angle between F' and F_A'' is θ ,

$$\cos \theta = \frac{|F(hkl)|^2 - |F(\bar{h}\bar{k}\bar{l})|^2}{4|F'| |F_A''|} \quad (4)$$

If the position of the anomalous scatterer is known, then F_A'' can be calculated.

The phase angle α of F' is

$$\alpha = \frac{\pi}{2} + \alpha_R \pm \theta.$$

Thus the phase angle values for all the (hkl) reflexions show significant intensity differences from their inverse $(\bar{h}\bar{k}\bar{l})$: Using this approach Ramachandran and Raman solved the phase problem in the case of *L*-lysine hydrochloride. Here the chlorine atom was used as the anomalous scatterer. It may be emphasized that the X-ray diffraction data were all measured using the indigenously constructed equipment. The remarkable success of the method encouraged many other crystallographers to employ it for solving the phase problem in more complicated organic molecules. Undoubtedly the anomalous dispersion method proposed and successfully employed for the solution of the phase problem for the first time is the most outstanding contribution of G N Ramachandran to crystallography. Indeed in the citation for the prestigious Ewald Prize awarded to him in the year 1999, his work on anomalous scattering is recognized.

Fourier Syntheses for Partially Known Crystal Structures

One of the earliest and successful methods of determining the three dimensional structure of molecules using the X-ray dif-

Box 3

The equation for the phase angle α define two possible solutions corresponding to $+\theta$ and $-\theta$. It is observed in practice that the final phase angle has a strong bias to lie close to the phase angle due to the anomalous scatterer.

fraction data is the so-called heavy atom method. When the crystal contains a relatively small number of heavy atoms – i.e. atoms with high atomic number – per unit cell, the X-ray scattering due to the heavy atoms will dominate the intensity of most of the Bragg reflexions. An electron density map $\rho(xyz)$, calculated with the structure amplitudes and using the phase angles based on the contributions of the heavy atoms would contain peaks at the heavy atoms positions in addition to a large number of peaks of smaller strengths corresponding to the positions of real atoms as well as a large number of spurious peaks. The problem is to identify the peaks corresponding to the real atoms against the equally strong ‘noise’ peaks. If some of the real atomic positions can be recognized, the phase angles can be recalculated using the extra structural information and the process repeated. The heavy atom method has been successfully used for solving complex molecule, the most outstanding one being the determination of the structure of vitamin B₁₂ by Dorothy Crowfoot Hodgkin (1957). Infact until the power of the ‘direct methods’ came to be established convincingly which took place after 1965, the heavy atom method was the most common method used by crystallographers. The justification for using the method was based on simple physical considerations and that it did work rather than any formal theoretical proof that the method should yield the unknown part of the crystal structure. Further nobody examined whether this was really the most efficient method for extracting the information from the known part nor did anyone investigate if there were other forms of Fourier synthesis which might produce the peaks of the unknown part better by ensuring a higher signal-to-noise ratio. Ramachandran raised these questions and examined them theoretically. His deep knowledge of the Fourier theory and principles of convolution was very valuable. He, with his students, developed many new forms of Fourier syntheses involving different theoretical combinations of the experimentally observed structure amplitudes $|F(hkl)|$, calculated structure factors ($F_{\text{cal}}(hkl)$) and phase angles. The different types of synthesis which they called α , β and γ syntheses, contain coeffi-

Box 4

Crystals in which the atomic positions are very nearly the same but differ only in the chemical nature of the atoms that occupy these positions are called isomorphous. The cell dimensions, crystal symmetry and the diffraction patterns of the isomorphous crystals are similar.



cients in the Fourier series as follows:

$$\begin{array}{ll} \alpha\text{-synthesis} & |F_{\text{obs}}|^2 F_{\text{cal}} = |F_{\text{abs}}|^2 |F_{\text{cal}}| e^{i\alpha}_{\text{cal}} \\ \beta\text{-synthesis} & |F_{\text{obs}}|^2 / F_{\text{cal}}^* = (|F_{\text{obs}}|^2 / |F_{\text{cal}}|) e^{i\alpha}_{\text{cal}} \\ \gamma\text{-synthesis} & |F_{\text{obs}}|^2 e^{i\alpha}_{\text{cal}}. \end{array}$$

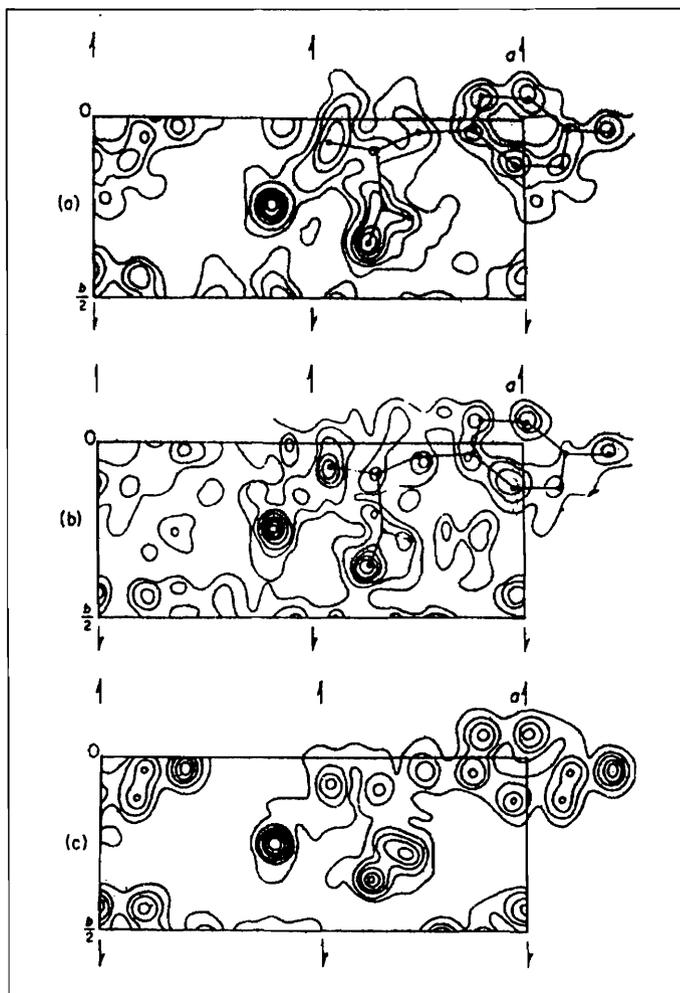
They found that the β -synthesis was the best in revealing the atomic positions of the unknown part with high signal-to-noise ratio. They also explored new forms of Fourier synthesis when anomalous dispersion data and isomorphous replacement data are available. The results of their research was published in a series of papers which led to the publication of an extremely valuable book entitled '*Fourier Methods in Crystallography*' (1970) by Ramachandran and R Srinivasan. One particular result reported by them is worth mentioning. They examined the nature of the Fourier synthesis using the correct phases, $\exp[i\alpha(hkl)]$ as coefficients. Interestingly, the map calculated showed peaks at positions of the correct structure as well as a few background peaks. They further calculated Fourier synthesis in which the magnitudes of all the structural factors are made equal but the correct phases are used as well as a synthesis in which the magnitudes of the structure factors were randomly distributed. The results observed were dramatic – in all the cases they observed the positions of the real atoms were the same as those in the ordinary Fourier synthesis (*Figure 2*). In other words, the phases really determine a structure and the magnitudes of the structure factors are only of secondary importance. It is important to mention that all these results were rationalised using convolution principles.

Intensity Statistics

The determination of the space group of a crystal is an important stage in the elucidation of the structure of a crystal by X-ray diffraction methods. A J C Wilson showed that the intensity data of the X-ray diffraction contain information regarding the presence or absence of a centre of symmetry. Making use of the central-limit theorem of statistics, he derived expressions for

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Figure 2. Non-centrosymmetric projection of tyrosine hydrochloride. (a) Phase synthesis. The magnitudes of all the structure factors were made equal, except for a decrease with $(\sin \theta)/\lambda$ corresponding to a mean atom, but the phases of the structure factors were correctly fed in. (b) Random synthesis. The magnitudes of the structure factors were randomly permuted among themselves, for each range of $(\sin \theta)/\lambda$. The phases were again correctly fed in. (c) Fourier synthesis. This corresponds to correct magnitudes and phases being fed in. Note the close similarity between the peaks in both (a) and (b) with (c) – in particular the fact that the chlorine atom shows up with a larger peak height than the other atoms. (From Srinivasan, 1961b.)



the distributions of the intensities for centrosymmetric and non-centrosymmetric crystals. His theory assumes that the crystal contains a large number of randomly distributed atoms of the same atomic number. Ramachandran and collaborators extended Wilson's basic work significantly and examined further a variety of crystallographic problems using crystallographic statistics. Suitable conditional joint probability distribution functions were developed using which they investigated the effect of the presence of atoms of dissimilar atomic numbers on the properties of intensity statistics of centrosymmetric and non-centrosymmetric crystals.

The success of the anomalous scattering method for structure determination depends on the possibility of achieving significant difference between the intensities (Bijvoet effect) of a large number of (hkl) and $(\bar{h} \bar{k} \bar{l})$ reflections. The Bijvoet effect would be expected to depend on the space group of the crystals, influence of the number of anomalous scatterers in the unit cell, influence of the effect of structural features such as the presence of local centre of symmetry in an otherwise non-centrosymmetric crystal and random positional errors of the anomalously scattering atoms. The influence of all the above factors were examined theoretically by Ramachandran and collaborators, particularly S Parthasarathy and R Srinivasan.

The reliability of the phases calculated using the heavy atom method depends on the magnitudes of heavy atom contributions and of the structure factor. It is possible to increase the signal-to-noise ratio in the electron density maps by applying suitable weighing criteria and this was studied by him. Criteria for testing the isomorphism of two crystals which when found are valuable to solve the phase problem were worked out using the conditional joint probability distribution functions. Although the theoretical studies carried out incisively by Ramachandran do not attract the attention of practising structural crystallographers, the significance of the results in bringing to light the subtle aspects of the processes involved in the problems investigated cannot be overemphasized.

Ramachandran would remain as one of the very few crystallographers in whose original contributions one witnesses intimate interplay between theory and experiment.

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Suggested Reading

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