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TOWARDS DIRECT PHASE RETRIEVAL IN MACROMOLECULAR CRYSTALLOGRAPHY

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Classical direct methods for x-ray structure determination (1) have made solution of the phase problem for small organic molecules relatively straightforward in most cases. However, these techniques (at least in their present forms) are not useful for the solution of large structures for two reasons. First, they are based on an atomicity property of the electron density that does not apply to macromolecules when diffraction data are not available at atomic resolution. Second, for large molecules, direct methods are unwieldy and unreliable.

Although phase problems in one dimension are inherently nonunique (2), recent theoretical work has shown that in two or more dimensions, the phase of the Fourier transform of a localized, positive function is uniquely determined by the amplitude (2–4). Furthermore, phase retrieval algorithms have been developed which are capable of reconstructing images from the magnitudes of their Fourier transforms with no phase information (5–7). Both the uniqueness properties and the phase retrieval algorithms depend on the amplitude being available continuously (in effect) in reciprocal space. Although this is the case in most imaging applications, in crystallography the amplitude is measured only at the reciprocal lattice points, so these results cannot be applied directly to crystallographic phase retrieval. To distinguish the former case from the later, we refer to them as "optical" (although this is not restricted to optics) and "crystallographic" phase problems. We describe here implications of the optical results for crystallographic phase retrieval and show how they may be incorporated into existing macromolecular phase retrieval algorithms to improve the convergence properties. This is a deterministic approach that is distinct from techniques based on maximum entropy.

THEORY

For ease of exposition, our discussion is restricted to two dimensions (as is usual in optical applications) and we consider crystal structures in the plane group P1 with a rectangular unit cell. Extension of the analysis and algorithms to three dimensions and to arbitary space groups is strightforward. The measured diffraction amplitudes are equal to the amplitude of the continuous Fourier transform of the electron density in a single unit cell, sampled at the reciprocal lattice points. The continuous intensity is the Fourier transform of the autocorrelation (8) of a single unit cell, which is identical to the Patterson of the density in an isolated unit cell. The linear extent of the autocorrelation is twice that of the unit cell so that, as a result of the sampling theorem (8), the continuous intensity (or amplitude) can be constructed from its samples only if they are separated by no more than half the spacing of the reciprocal lattice points. Hence, to make use of the optical results, amplitude measurements must be available on a grid with a spacing no greater than this. We call the amplitudes at the reciprocal lattic points the "ordinary" structure amplitudes and others, which are on a grid with half the spacing, the "inbetween" structure amplitudes (Fig. 1). Knowledge of both the ordinary and inbetween structure amplitudes then is equivalent to knowing the continuous amplitude.

Using the continuous amplitude, optical *ab initio* phase retrieval involves two steps. First, approximate phases are generated from the ordinary and inbetween amplitudes using a noniterative procedure (3) that we call "crude phase estimation. " The second step involves iteratively improving these phases by iterating between real space and reciprocal space, forcing the image to conform to any a priori information in the former, and forcing the calculated amplitudes to be equal to those measured in the latter. The iterative procedure is often called a "Fienup algorithm" (5, 7) in optics and typical constraints on the image are positivity and known extent. Equivalent procedures (referred to as density modification) are used in crystallography, and typical constraints in real space are positivity, known molecular boundaries, and equivalence of identical subunits (9, 10). Application of the Fienup algorithm in

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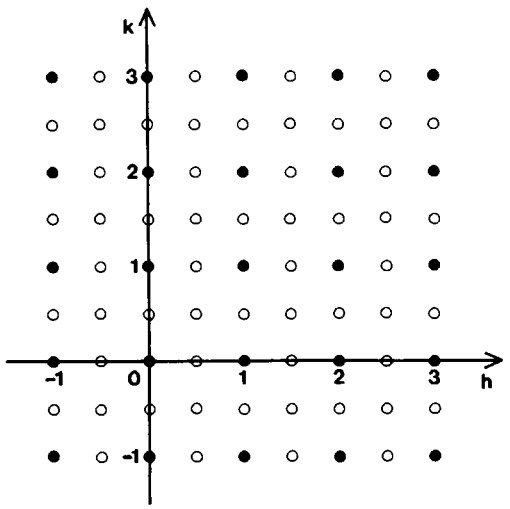

FIGURE 1 Two-dimensional reciprocal space (h and k are the Miller indices) showing positions of the ordinary (●) and inbetween (○) structure amplitudes.

optics usually produces successful convergence even when started with random initial phases (5) although convergence can be slow. Starting with the crude phase estimates can substantially reduce the number of iterations required (7).

Noncrystallographic symmetry occurs in many macromolecules such as proteins and viruses that are composed of identical subunits (9). The possibility of using noncrystallographic symmetry for *ab initio* phase determination was recognized in the early 1960s (cf., Argos and Rossmann (9) and the references they cite) but it has been found useful primarily for phase refinement and extension by real space averaging (9, 10). Noncrystallographic symmetry allows the continuous amplitude to be determined at positions between the reciprocal lattice points, and we call these "inferred" structure amplitudes. If a structure contains sufficient noncrystallographic symmetry, there may be enough over-sampling in reciprocal space for the inbetween amplitudes to be estimated reasonably accurately.

PHASE RETRIEVAL ALGORITHM

Point group rotational noncrystallographic symmetry is the most common and leads to rotational symmetry in reciprocal space so that determination of the positions and magnitudes of the inferred structure amplitudes is very easy. Hence, we consider here a simple two-dimensional structure with N-fold noncrystallographic symmetry. The molecule is confined to a circular envelope (centered on the rotation axis) in which the noncrystallographic symmetry is valid. The autocorrelation then has $2N$-fold symmetry and is confined to a circular envelope of twice the radius. Each inbetween structure amplitude initially is equated to the nearest inferred structure amplitude. An initial autocorrelation is then calculated which is averaged to enforce the $2N$-fold symmetry and set to zero outside the autocorrelation envelope. This autocorrelation is Fourier-transformed to calculate an improved set of inbetween structure amplitudes that are combined with the measured ordinary structure amplitudes to calculate a new autocorrelation. This procedure is repeated until stable estimates of the inbetween structure amplitudes are obtained. Obviously the accuracy of the inbetween amplitudes so obtained increases with increasing N. Crude phase estimates can then be obtained for all the structure factors (3, 6). Iterative density modification is then performed in the usual manner starting with these phases except that we use the inbetween as well as the ordinary structure amplitudes to use the continuous amplitude information. Once the phases have stabilized, they will be accurate enough (hopefully) so that when the inbetween structure amplitudes are removed (because they do not constitute genuine data), further phase refinement using the ordinary structure amplitudes alone, as in conventional density modification, will produce convergence to the correct solution.

RESULTS

To study the convergence properties of the above algorithm, data to 2.5 Å resolution were generated for a two-dimensional model structure (Fig. 2) in which the threefold symmetry, location of the rotation axis and envelope are assumed known. Phase retrieval was performed with the modified procedure described above using the inbetween amplitudes in the first cycle only. Phase retrieval was also performed using conventional density modification (electron density averaging and solvent leveling) starting from random phases and using only the ordinary structure amplitudes. The crystallographic residuals vs. iteration are shown in Fig. 3. Our modified procedure converges faster than the conventional method. When the amplitude data are incomplete and noisy, the

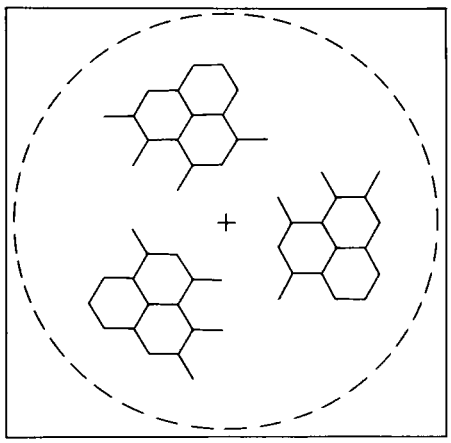

FIGURE 2 The two-dimensional model structure. The unit cell is square with dimensions 23 Å and contains three subunits related by a noncrystallographic threefold axis. The structure consists of carbon and oxygen atoms, each represented by an appropriate Gaussian electron density function. The broken line denotes the envelope.

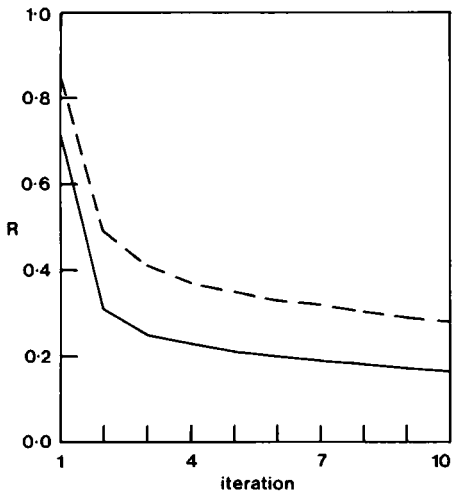

FIGURE 3 The crystallographic residual (R) vs. iteration for the phase retrieval procedure described here (—) and for conventional density modification (---).

improved performance could make the difference between successful and unsuccessful convergence.

DISCUSSION

Theoretical results for the phase problem in optics and the modifications to crystallographic phase retrieval algorithms described here have a number of general implications for the crystallographic phase problem.

The theoretical results indicate that knowledge of the continuous amplitude is sufficient for the phases to be uniquely determined, at least for positive images. To assure a unique solution, additional information must be sufficient to allow the continuous amplitude to be constructed. For example, geometric redundancy must be at least eightfold (in three dimensions) to ensure uniqueness in the absence of any other *a priori* information except positivity. In practice however, a variety of other information, such as molecular envelopes, is usually available, which decreases the redundancy required. The redundancy also must have substantial components in all directions in reciprocal space to allow construction of the continuous amplitude in all directions.

Implementing crude phase estimation may be difficult with large unit cells because of the difficulty of measuring reflections close to the beam stop. However, the amplitudes and phases of low-resolution reflections can sometimes be measured using techniques such as solution scattering combined with low-resolution modeling, or electron microscopy. These low-resolution phases could then be extended to higher resolution using crude phase estimation. Solution of the "translation problem" (9) still presents difficulties when no independent phase information is available, although with modern computers "brute force" R-factor and steric searching are feasible.

Inclusion of the inbetween amplitudes in phase refinement by density modification may be important since the continuous amplitude information is then utilized when the phase errors are large and convergence towards the correct solution is more difficult. This may, for example, improve convergence from noisy isomorphous replacement phases when conventional refinement fails. We are currently investigating the significance of this.

Finally, structural hierarchy (11) and the presence of voids in most macromolecules probably impose phase constraints, in the same way that atomicity does at high resolution. This may allow the extension of direct methods to macromolecular crystallography, as has recently been proposed (11).

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