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Ab Initio Phase Determination for X-ray Diffraction Data from Crystals of a Native Protein

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Abstract

An efficient algorithm for the determination of an everywhere positive electron-density distribution that agrees with observed structure amplitudes has been used to determine the phases of X-ray diffraction data from recombinant bovine chymosin, a protein with 323 amino-acid residues in the molecular chain whose structure was recently determined using molecular replacement methods. A systematic procedure for testing the signs of centric reflections, using the total entropy of the map as a figure of merit, was used to produce a low-resolution map. The phases of acentric and additional centric reflections were then chosen by adding them to the map with various possible phases and computing the total entropy of the resulting map. Of 159 centric reflections whose phases were chosen by this procedure, 141 had the same phase as in the refined structure. The median absolute phase difference for 1811 acentric reflections was 32°. A map produced from these 1970 reflections, out of 12 346 reflections in the data set, showed a remarkable agreement with the refined structure. This molecule is many times larger than any whose structures have previously been determined without the use of isomorphous replacement, molecular replacement or anomalous dispersion, and the map demonstrates the potential of maximum-entropy methods in macromolecular structure determination.

Introduction

Direct methods of phase determination rely on the fact that, although diffraction intensities are proportional to the squared moduli of the structure factors, which are the complex values of the Fourier transform of the electron density in the unit cell, and thus contain no phase information, the non-negativity of the electron-density places restrictions on the possible values of the phases. Karle & Hauptman (1950) expressed these restrictions in the form of determinantal inequalities, which imply that the modulus of the difference between a structure factor and another complex number that is a function of other

structure factors must be less than or equal to a positive real number that is also a function of other structure factors. Hauptman & Karle (1950) and Goedkoop (1950) also showed that, for a distribution of N point atoms, determinants larger than $N \times N$ must be equal to zero, leading to exact relations among large sets of structure factors. For all but the simplest crystal structures N is too large for practical use and direct methods have therefore been developed around probability relations among much smaller sets of structure factors. [For reviews of the development of direct methods, see Karle (1985) and Woolfson (1987).] The existence of these relations implies, however, that, if an everywhere non-negative distribution can be found, the amplitudes of whose Fourier coefficients are equal to the observed amplitudes for a sufficiently large set of reflections, the amplitudes and phases of all structure factors must be determined to an arbitrary precision.

Maximum entropy is one of a class of density-modification techniques that are related to direct methods. Much attention has recently been directed toward this technique, not only by crystallographers but also by investigators in branches of physics such as spectroscopy and radio astronomy (Jaynes, 1979; Gull & Daniell, 1978; Collins, 1982; Collins & Mahar, 1983; Wilkins, Varghese & Lehmann, 1983; Livesey & Skilling, 1985). Bricogne (1984) has given an extensive analysis of maximum-entropy methods and he has shown that, in theory, they should be powerful enough to produce robust phase solutions. Previous applications have, however, suffered from the lack of an efficient numerical procedure for finding the maximum-entropy solution. Thus either the computations have been tedious or the approximations have been too crude to produce a significant enhancement of phasing power.

The principle of maximum entropy defines distribution functions that ensure non-negativity. Although many other properties of maximum-entropy distributions are discussed in earlier papers, the non-negativity property is the only one that is used in this work, so that the methods described in this paper are maximum-entropy methods only in the very limited sense that they make use of the constrained maximum of a function. In particular, no use is made of the properties of maximum entropy that are derived from information theory. Collins & Mahar (1983), using a maximum-entropy argument, proposed an exponential model for electron density, with iterative adjustment of the logarithms of the density in order to get a better approximation to the structure amplitudes, but they did not give a complete solution to the problem of finding an exact fit. Other attempts, such as those of Wilkins (1983), Navaza (1985) and Gull, Livesey & Sivia (1987), to find maximum-entropy probability distributions with the constraint of satisfying observed Fourier amplitudes have not been readily applicable to large-scale problems. Recently, Bricogne & Gilmore (1990) and Gilmore, Bricogne & Bannister (1990) have described a procedure in which likelihood is combined with maximum entropy that has shown considerable promise for application to problems involving several hundred atoms, but this procedure has not been applied to large-scale problems.

The numerical problem of finding an exponential model that satisfied experimental constraints was addressed by Prince, Sjölin & Alenljung (1988) and by Prince (1989), who showed that the logarithmic space coefficients could be determined to an arbitrary precision using an iterative procedure similar to nonlinear least squares involving a positive-definite matrix. This matrix can be shown (Luenberger, 1984) to be the Hessian matrix of a dual function whose unconstrained minimum is equal to the constrained maximum of the entropy function, which suggests using the entropy of the map as a figure of merit for comparing different maps that satisfy the non-negativity condition and some number of Fourier amplitudes. In this paper we describe a systematic procedure for finding phases that produce an everywhere positive map whose Fourier amplitudes agree with the observed data, using the entropy of the map to choose among different maps with the same quality of agreement, and a test of the procedure in which the map is compared with a known refined structure.

Mathematical analysis

Consider a unit cell divided into n subunits, commonly called pixels. In maximum entropy we seek the maximum of

$$S = -\sum_{k=1}^{n} \rho_k \ln \rho_k, \qquad (1)$$

subject to the system of constraints

$$\left| \sum_{k=1}^{n} \rho_k \exp(2\pi i \mathbf{h}_i . \mathbf{r}_k) \right| = |F_{\mathrm{obs}}(\mathbf{h}_j)| \qquad (2)$$

for a set of m reflections. Here ρ_k is the number of electrons in the pixel located at $\mathbf{r}_k$ and $\mathbf{h}_j$ is the vector of Miller indices for reflection j. It can be shown (Luenberger, 1984) that the constrained maximum of S is equal to the unconstrained minimum of its dual function, which is defined by

$$\Phi(\mathbf{x}) = -\sum_{k=1}^{n} \rho_k(\mathbf{x}) \ln \rho_k(\mathbf{x})$$

$$+ \sum_{j=1}^{m} x_j [|F_{\mathrm{map}}(\mathbf{h}_j, \mathbf{x})| - |F_{\mathrm{obs}}(\mathbf{h}_j)|], \qquad (3)$$

where

$$F_{map}(\mathbf{h}_j, \mathbf{x}) = \sum_{k=1}^{n} \rho_k(\mathbf{x}) \exp(2\pi i \mathbf{h}_j \cdot \mathbf{r}_k), \qquad (4)$$

$$\rho_k(\mathbf{x}) = \exp\left[\sum_{j=1}^{m} x_j \cos(2\pi \mathbf{h}_j \cdot \mathbf{r}_k - \varphi_j)\right], \qquad (5)$$

φ_j is a phase for reflection j inferred from any available prior information, and $\mathbf{x}$ is a vector of parameters that may be identified with the Lagrange multipliers of more conventional constrained optimization. $\rho_k(\mathbf{x})$ is clearly non-negative, and because the argument of the exponential in (5) is itself a Fourier series, is periodic, so that it can be expanded in a Fourier series that will contain nonzero amplitudes, and also phases, for terms other than those contained in the argument.

In order to apply efficient numerical methods to finding the minimum of a function of degree higher than quadratic, it is necessary (Gill, Murray & Wright, 1981; Luenberger, 1984; Prince, 1982) to have an expression for its gradient and at least an approximation to its Hessian matrix. The gradient of $\Phi(\mathbf{x})$ is

$$\nabla[\Phi(\mathbf{x})]_j = |F_{map}(\mathbf{h}_j, \mathbf{x})| - |F_{obs}(\mathbf{h}_j)|, \qquad (6)$$

so that it vanishes when the constraint conditions are satisfied. The Hessian matrix of $\Phi(\mathbf{x})$ is (Prince, 1989)

$$\mathbf{H}[\Phi(\mathbf{x})] = \mathbf{CPC}^T, \qquad (7)$$

where

$$C_{jk} = \cos(-2\pi \mathbf{h}_j \cdot \mathbf{r}_k + \varphi_j), \qquad (8)$$

and $\mathbf{P}$ is a diagonal matrix with $P_{kk} = \rho_k$. $\mathbf{H}[\Phi(\mathbf{x})]$ is everywhere positive definite, so that, for any set of initial phases, φ_j, $\Phi(\mathbf{x})$ has at most one minimum. Note that if the assumed value of $F(000)$ is not large enough, there will be no minimum, so that the entropy will be negatively infinite. If a minimum does exist, the second sum in (3) vanishes, and the value of the dual function is the entropy of the fitted map, which can then be used as a figure of merit for comparing various choices of values of the initial phases.

A typical element of the Hessian matrix is

$$H_{ij} = \sum_{k=1}^{n} \rho_k(\mathbf{x})[\cos(2\pi \mathbf{h}_i \cdot \mathbf{r}_k - \varphi_i)$$

$$\times \cos(2\pi \mathbf{h}_i \cdot \mathbf{r}_k - \varphi_j)], \qquad (9)$$

which, because

$$\cos \alpha \cos \beta = [\cos(\alpha + \beta) + \cos(\alpha - \beta)]/2,$$

reduces to

$$H_{ij} = \{|F_{map}(\mathbf{h}_i + \mathbf{h}_j)| \cos[\varphi_{i+j} - (\varphi_i + \varphi_j)]$$

$$+ |F_{map}(\mathbf{h}_i - \mathbf{h}_j)| \cos[\varphi_{i-j} - (\varphi_i - \varphi_j)]\}/2. \qquad (10)$$

If structure factors are computed using a fast Fourier transform (FFT) algorithm, all quantities required in

a computation of the Hessian matrix are computed at one time, independent of the number of reflections being fitted in a block.

Because the Hessian matrix is positive definite, the minimum of the dual function can be found using standard numerical methods. The method adopted for this work is a quasi-Newton method (Gill, Murray & Wright, 1981) with a line search to ensure convergence. In this method the Hessian is computed and factored once. When the line search has found a point at which the line derivative of the dual function is sufficiently reduced, the position of that point in parameter space and the gradient at that point are used to compute a numerical correction to the Hessian (in practice it is applied to the Cholesky factor of the Hessian) that would have predicted the value of the gradient actually found at that point. This corrected Hessian matrix is then used in the next line search. The correction formula that has been found to be most satisfactory is known as the Broyden–Fletcher–Goldfarb–Shanno, or BFGS, update. The stopping rule requires that all calculated amplitudes be equal to the observed amplitude within a tolerance that is set so that the variation of the entropy of the map within the acceptance volume is small compared with the variations resulting from changing the input phases.

Because a Fourier expansion of the density function given in (5) contains terms in addition to those used in its construction, the fitting procedure can be used in a straightforward manner to extend phases to additional reflections, and fitting the amplitudes of the new reflections helps to refine the phases of previously included ones. Therefore, if a substantial sized starting set of phases can be obtained, by isomorphous or molecular replacement, for example, these methods can be used to extend phases to higher resolution. If, however, there is no previously available phase information, a different strategy is necessary.

In nonpolar space groups there are sets of reflections in all three dimensions whose phases are constrained by symmetry to have one of two values. The phases of one, two or three reflections, depending in the space group, may be chosen arbitrarily to define the origin. In a few space groups the phase of one additional reflection may be chosen arbitrarily to define an enantiomorph. A possible strategy would be to compute an initial entropy-maximized map using up to four of these centric reflections, and then add reflections one at a time, in descending order of $|F|$, determining the maximum-entropy fit for each possible phase, and choosing the phase for which the resultant map had higher entropy. This simple strategy might be subject to difficulties if the effects on entropy of different reflections are not strictly additive, but might depend on the order in which additional reflections are introduced. The risk of

encountering such problems can be reduced by trying all four combinations of two signs, or all eight combinations of three signs, in a so-called 'full factorial design' (Box, Hunter & Hunter, 1978), but the number of combinations that must be tried increases rapidly as the number of reflections added in a block increases.

Another alternative strategy is to use a 'fractional factorial design' (Box, Hunter & Hunter, 1978) in which a systematically chosen subset of the possible sign combinations is used. The points in a 16-point fractional factorial design give 32 sign combinations that are the positive and negative senses of 16 mutually orthogonal vectors, so that there are 16 entropy differences, one for each pair. A 16-point fast Fourier transform, known in the statistical literature as 'Yates's algorithm' (Box, Hunter & Hunter, 1978), is used to extract estimates of the effects of the individual reflections. Use of this procedure does not assume that there are no effects of multiple sign changes, or even that the single changes are dominant, but only that the various multiple changes that are confounded with each single change tend to average out. The procedure that has been adopted for this work is to add reflections in blocks of 16, using the 32 sign combinations in the fractional factorial design, the one additional one indicated by Yates's algorithm, and 16 more generated by single sign changes from the previous highest entropy, for a total of 49 trial sets.

In space groups that contain nonintersecting axes, the structure factors of some of the centric reflections are imaginary. Maximum-entropy maps constructed from centric reflections in these space groups are consequently enantiomorphous and, when a sufficient number of centric reflections has been inserted, a Fourier inversion of the map will show some acentric reflections that have significant calculated amplitudes, with general phases. Phase extension can then proceed by inserting those reflections with those phases and applying the fitting algorithm to fit their amplitudes. In space groups, such as $I222$, that do not contain nonintersecting axes, the structure factors of all centric reflections are real and therefore contain no enantiomorph-determining information. Phase determination for acentric reflections must begin by trying many phases around the circle for a strong reflection and determining what trial phase leads to maximum entropy. For the first acentric reflection added the entropy will be symmetrical about 0 and π, reflecting the lack of enantiomorph information, and one maximum may be chosen. Thereafter there should be a unique maximum around the circle, but it may be necessary to determine the phases of many reflections by this rather tedious procedure before other acentric reflections appear with significant calculated amplitudes, so that phase extension can proceed automatically.

Test of *ab initio* phase determination

As a test to determine whether maximum-entropy methods might be useful for *ab initio* structure determination, a program for generating maximum-entropy maps using this procedure has been applied to the determination of phases in order to generate an electron-density map of the previously solved structure of recombinant bovine chymosin (Gilliland, Winborne, Nachman & Wlodawer, 1990). This protein contains 323 amino-acid residues and crystallizes in space group $I222$ with unit-cell dimensions $a = 72 \cdot 7$, $b = 80 \cdot 2$ and $c = 114 \cdot 8$ Å. There is one molecule in the asymmetric unit. In this space group, the structure factors of all $hk0$, $h0l$ and $0kl$ reflections must be real. The phase of one reflection from each set may be chosen arbitrarily to define the origin. A strong reflection was therefore selected from each set and, to ensure that the origin of the map would correspond to that of the refined structure, assigned the phase that it had in the refined structure, and an initial map was calculated using a conventional Fourier synthesis program and converted to a maximum-entropy map whose Fourier amplitudes matched the observed amplitudes of these three reflections by the method described by Prince (1989). Next, a set of 16 strong reflections was chosen from the three special sets and 32 of the total 2^{16} possible sign combinations were chosen according to a 16-point fractional factorial design. Maximum-entropy maps were computed with each sign combination and the total resulting entropy for each map was determined. From this it could be determined, using the methods described above, which set of signs produced the highest entropy. 48 additional reflections were added, again in sets of 16, by the same procedure.

In this space group there are no reflections whose structure factors are constrained by symmetry to be imaginary, so that it is necessary to use a general hkl reflection to define the enantiomorph. When the phases of 67 centric reflections had been determined, therefore, the strongest acentric reflection, 7,13,14, was selected and its amplitude fitted by maximum entropy with its phase set at each multiple of 20° from 0 to 340°. Fig. 1 shows the total entropy of the fitted map, plotted against the phase angle. The curve is symmetric about π, with equal maxima at 60 and 300°. Because the phase of this reflection in the refined structure was 64°, the 60° maximum was chosen. (It should be noted that this phase is completely arbitrary. Nothing in the input requires that it be anywhere near either maximum.) A similar plot was then calculated for the next-strongest acentric reflection, 9,1,20, with extra points determined in the vicinity of the maximum, with the result shown in Fig. 1. The plot is no longer symmetrical and it has a unique maximum at 316°. The phases of 27 additional reflections were then determined using the same procedure.

For these 29 reflections, the average difference between the phases determined by this procedure and the phases calculated from the refined structure was about 20°.

On the assumption that most information is contained in those reflections whose observed and calculated intensities differ the most, and considering that the largest differences from a uniform distribution are the largest amplitudes, reflections were added to the map in descending order of $|F|$. After 29 reflections had been phased by hand, the procedure was automated by having the computer test each initial phase for each reflection from a list of the strongest ones, using the map computed from 67 centric reflections and 29 acentric reflections as a prior. It was realized that this procedure could be followed in parallel by several computers with different lists of reflections, although each would necessarily have to work with the same prior map. Therefore, ten different MicroVAX II* computers were assigned lists of 100 reflections each and, after several days of computing, a list of 948 reflections was assembled. This procedure was repeated and, after several more days, a final electron-density map was calculated using 159 centric and 1811 acentric reflections out of a total of 12 346 reflections to 1·6 Å resolution. Of these 1970 reflections, 1809 had resolutions in the range 2·0–8·0 Å and 36 had resolutions greater than 2·0 Å. In performing these computations, no effort was made to optimize the computer programs. It is therefore possible to increase the computing speed by a large factor, particularly by introducing FFT routines for structure-factor and map calculations.

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* MicroVAX II is a trademark of the Digital Equipment Corporation. Trade names are identified in order to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the product is the best available for the purpose.
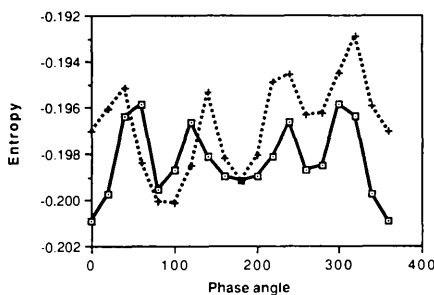

Fig. 1. The maximum extropy of a fitted electron-density map, plotted as a function of the initial phases of the two strongest acentric reflections. For the solid line the prior map contains only centric reflections and the 7,13,14 reflection has been added. The plot is symmetric about π, reflecting the two possible enantiomorphs. For the dotted line the prior contains the 7,13,14 reflection and the 9,1,20 reflection has been added. The plot is no longer symmetrical.

These calculations are always done with the phases of the Fourier terms that appear within the argument of the exponential in (5) fixed. A question of particular interest is to what extent can the phases change in the course of the calculation. In any given cycle, fitting of the amplitudes of a small subset, up to a few hundred, of reflections affects the structure factors, both amplitude and phase, of all reflections, because of the lack of orthogonality in the exponential map. Thus, the phases of acentric reflections can be adjusted throughout, but centric reflections, except for weak ones that are entered late in the calculation, will always have the same phases.

Results

A comparison of the phases computed by inversion of the final map with those from the refined structure showed that 141 of the centric reflections had the same phases, while the median absolute phase difference for the acentric reflections was 32° and 90% of them had differences less than 77°. This may be compared with the differences observed in a small-molecule structure determination when the direct-methods program in the structure determination package *SHELX*86 (Sheldrick, 1986) is used to determine phases. In a recent structure solution of an organic molecule with the chemical formula $C_{28}H_{46}O_3$ (Koceovsky, Langer & Gogoll, 1990), the phases estimated from 500 reflections were compared to the final phases after the structure had been refined to an unweighted R of 0·041. The median phase difference for the 410 acentric reflections in this set is 20·6°.

The remarkable agreement between the calculated map and one calculated with phases from the previously refined structure is illustrated in Fig. 2, which shows maps calculated with the *FRODO* (Jones, 1978) graphics system. In Fig. 2(a), with 948 reflections, there are still substantial gaps in the density. Fig. 2(b), with 1970 reflections, shows a region of the map that is almost indistinguishable from Fig. 2(c), which is calculated using all data with phases from the refined structure. (Again it should be noted that, except for fixing the origin and the enantiomorph, there are no degrees of freedom in the calculation of the maximum-entropy map.) In the 323-residue length of the chain, there are 28 gaps, all of them less than 3·5 Å wide. In the map for the refined structure there are seven gaps, particularly in the so-called 'flaps' region (Gilliland *et al.*, 1990). Within a molecular envelope whose border was placed one van der Waals's radius from the surface atoms, there is virtually no density that is not associated with the protein molecule or a water molecule in the first shell of hydration. Outside the envelope, density occurs only in isolated clumps.

Contrast is an important factor in macromolecular structure analysis. When too small a set of reflections

is used, as in our 948 reflection map, the contrast tends to be poor and the molecular envelope poorly defined. In the 1970 reflection map the protein is clearly visible and, since extra density in the bulk solvent region occurs only in isolated clumps, the contrast is sufficient for practical purposes.

Discussion

One goal of this study was to determine whether a set of centric reflections from a macromolecular crys-
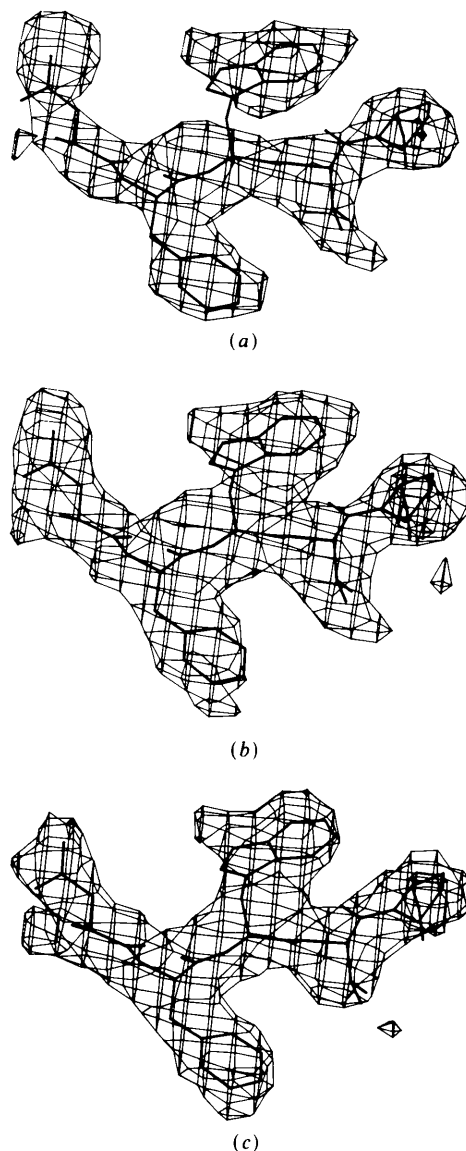

(a)

(b)

(c)

Fig. 2. Portions of three density maps, compared with a four amino-acid-residue segment of the molecule of recombinant bovine chymosin. (a) A map computed from 948 reflections, phased by maximum entropy. (b) The map with phases extended by maximum entropy to 1970 reflections. (c) The corresponding map computed using all data with phases calculated from the refined structure (Gilliland et al., 1990).

Table 1. *The sign combinations in a* 16-*point fractional factorial design giving* 32 *sign combinations*

For each sign combination, the value of the entropy is shown for $F(000)$ equal to the correct value, twice the correct value and half the correct value.

Sign combinations for the 16 strongest reflections	Calculated entropy		
	Correct	Twice $F(000)$	Half
+++++++++++++++++	−0·101594	−0·101603	−0·101718
−−−−−−−−−−−−−−−−	−0·101607	−0·101616	−0·101615
+−+−+−+−+−+−+−+−	−0·101575	−0·101618	−0·101632
−+−+−+−+−+−+−+−+	−0·101618	−0·101600	−0·101705
++−−++−−++−−++−−	−0·101598	−0·101616	−0·101670
−−++−−++−−++−−++	−0·101609	−0·101593	−0·101669
+−−++−−++−−++−−+	−0·101569	−0·101597	−0·101669
−++−−++−−++−−++−	−0·101577	−0·101609	−0·101647
++++−−−−++++−−−−	−0·101598	−0·101613	−0·101645
−−−−++++−−−−++++	−0·101593	−0·101611	−0·101645
+−+−−+−++−+−−+−+	−0·101590	−0·101655	−0·101598
−+−++−+−−+−++−+−	−0·101633	−0·101627	−0·101601
++−−−−++++−−−−++	−0·101572	−0·101631	−0·101645
−−++++−−−−++++−−	−0·101658	−0·101587	−0·101685
+−−+−++−−+−++−−+	−0·101606	−0·101622	−0·101669
−++−+−−+−++−+−−+	−0·101588	−0·101600	−0·101634
++++++++−−−−−−−−	−0·101598	−0·101626	−0·101645
−−−−−−−−++++++++	−0·101615	−0·101605	−0·101668
+−+−+−+−−+−+−+−+	−0·101625	−0·101591	−0·101641
−+−+−+−++−+−+−+−	−0·101556	−0·101611	−0·101630
++−−++−−−−++−−++	−0·101620	−0·101593	−0·101665
−−++−−++++−−++−−	−0·101582	−0·101562	−0·101686
+−−++−−+−++−−++−	−0·101575	−0·101585	−0·101634
−++−−++−+−−++−−+	−0·101595	−0·101624	−0·101687
++++−−−−−−−−++++	−0·101614	−0·101596	−0·101616
−−−−++++++++−−−−	−0·101570	−0·101604	−0·101613
+−+−−+−+−+−++−+−	−0·101585	−0·101612	−0·101667
−+−++−+−+−+−−+−+	−0·101622	−0·101604	−0·101634
++−−−−++−−++++−−	−0·101604	−0·101634	−0·101662
−−++++−−++−−−−++	−0·101609	−0·101615	−0·101671
+−−+−++−+−−++−−+	−0·101589	−0·101587	−0·101637
−++−+−−+−+−++−+−	−0·101574	−0·101597	−0·101623
Correct signs			
+−+++−−+++−+−+−+	−0·101530	−0·101550	−0·101589

tal could be phased reliably by using maximum entropy as a figure of merit. Using a fractional factorial design to limit the number of sign combinations that had to be tried, 138 out of 156 phases (88%) that were determined by the maximum-entropy procedure were the same as in the refined structure. Earlier tests of the algorithms on selected known simpler structures had suggested that the phasing accuracy could be in the range 80 to 93%. The high accuracy with which, at least in favorable conditions, the phases of subsets of centric reflections can be determined is in itself an important result of this study. One consequence of this is that, in phase determination using single isomorphous replacement, the phases of centric reflections in the native data can be determined independently, and a difference map can then be computed that shows the positions of the heavy atoms only. This could be particularly useful if the Patterson map, for various reasons such as multiple heavy-atom sites, was difficult to interpret.

Another goal of this study was to determine if the phases of acentric reflections, particularly in unfavorable space groups like I222, could be determined by

Table 2. *The* 16 *single sign changes for the correct* F(000), *twice the correct* F(000) *and half the correct* F(000)

Sign combinations and the associated entropy term for					
Correct F(000)		Twice the F(000)		Half the F(000)	
---++--++--++--+	-0·101581	+-++--++++--++--	-0·101609	--+--+-++-+--+-+	-0·101639
++-++--++--++--+	-0·101570	-+++--++++--++--	-0·101578	+++--+-+++-+--+-+	-0·101637
+-+++--++--++--+	-0·101580	---+--++++--++--	-0·101602	+----+-++-+--+-+	-0·101635
+---+--++--++--+	-0·101580	--+---++++--++--	-0·101599	+-++-+-++-+--+-+	-0·101622
+--+---++--++--+	-0·101602	--+++-++++--++--	-0·101579	+-+-++-++-+--+-+	-0·101603
+--+++-++--++--+	-0·101581	--++-+++++--++--	-0·101606	+-+----++-+--+-+	-0·101620
+--++-+++--++--+	-0·101575	--++--+++*-++--+	-0·101583	+-+--+-++++-+--+-+	-0·101638
+--++---+--++--+	-0·101576	--++--+-++--++--	-0·101617	+-+--+--+-+--+-+	-0·101641
+--++--+---++--+	-0·101614	--++--++-+--++--	-0·101587	+-+--+-+--+--+-+	-0·101648
+--++--+++-++--+	-0·101593	--++--+++---++--	-0·101588	+-+--+-+++-+--+-+	-0·101649
+--++--++-+++--+	-0·101598	--++--+++++--++--	-0·101614	+-+--+-++----+-+	-0·101655
+--++--++---+--+	-0·101612	--++--++++-+++--	-0·101564	+-+--+-++-++-+-+	-0·101629
+--++--++--+---+	-0·101586	--++--++++---+--	-0·101584	+-+--+-++-+-++-+	-0·101592
+--++--++--+---+	-0·101550	--++--++++--+---	-0·101618	+-+--+-++-+--+--+	-0·101618
+--++--++--++-++	-0·101601	--++--++++--+++-	-0·101612	+-+--+-++-+--++++	-0·101620
+--++--++--++---	-0·101625	--++--++++--++-+	-0·101611	+-+--+-++-+--++-+	-0·101625

Best signs are +--++--++--+++-+ entropy = -0·101550 for the correct F(000)
Best signs are --++--++++-+++-- entropy = -0·101564 for twice the F(000)
Best signs are +-+--+-++-+-++-+ entropy = -0·101592 for half the F(000)

systematic trials of various initial phases. If this could be done, the problem would be reduced to one similar to the one for centric reflections, except that the number of trials would be much greater. That is a matter of computing power, not one of having to make simplifying approximations of uncertain validity.

An additional implication of these results is that the maximum-entropy procedure can extend phases rather accurately. Low-resolution phases of good quality can often be obtained by isomorphous or molecular replacement, but lack of isomorphism and similar effects cause extension to higher resolution to be unreliable. The phasing power of maximum-entropy fitting increases as the amount of information incorporated in the prior map increases, so that further additional phases are potentially more and more accurate.

A potential problem in solving an unknown structure is the proper value of F(000). In the case described here the scale was effectively inferred from the solved structure. In an unknown structure, too small a value of F(000) will cause the phase-extension process to diverge, because the entropy of the map that fits everything is negatively infinite. Too large a value, on the other hand, may cause the tests to lose discriminating power.

The effect of the value of F(000) was studied by repeating the measurements of the entropy for the various sign combinations for the first block of sixteen reflections with this value set to one half and twice its correct value. Tables 1 and 2 show the results of this calculation.* Whereas 13 of the 16 signs were correct, when compared with those from the refined structure, for the correct F(000), only eleven were correct for the doubled F(000), and only nine were correct for the half F(000). However, it was found that the correct set of signs had, by a substantial

amount, the highest entropy in all three cases, although the procedure followed failed to find it. It appears, therefore, that a more-extensive search procedure should be used, although it is not clear how many of the initial phases need to be correct in order to lead to a recognizable density map.

In order to determine the effect of an incorrect value of F(000) on the determination of the phases of acentric reflections, the phases of the first two of these reflections were also determined using half and twice the correct value. The entropies differed from those shown in Fig. 1 only in the fifth decimal place. Thus it appears that, once a prior distribution contains a reasonable number of centric reflections, the phases of acentric reflections are insensitive to the value of F(000).

Further analysis of these results suggests that a map based on approximately 15% of the data is needed to produce a map from which the proper connectivity of the chain can be satisfactorily determined. Even if the contrast between the molecule and the solvent is lower with this fraction of the data, it is still, at least in this case, quite sufficient. The probability that a map of the clarity of this map of recombinant bovine chymosin, and phases in comparable agrement with those from the solved structure, would be produced by a random process would appear to be extraordinarily small. The indication is, therefore, that the total entropy of a map is an extremely powerful figure of merit for the choice of phases in macromolecular structures.

The largest structures that have been determined using classical direct methods have had a few hundred atoms in the asymmetric unit. Using a maximum-entropy method different from the one described here, Harrison (1989) solved a macromolecular structure with 610 atoms in the asymmetric unit. The structure of recombinant bovine chymosin, with 2808 non-H

atoms in the asymmetric unit, is almost a factor of five larger than any structure that has previously been determined without the use of isomorphous or molecular replacement or anomalous dispersion.

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The Reflected and Refracted Fundamental Modes of Dynamical X-ray Diffraction

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Abstract

An energy-conservation relation is derived between the power absorption, energy flux and absorption coefficient of an arbitrary fundamental mode in the n-beam dynamical theory of X-ray diffraction. From this relation, it is proven that the $4n$ fundamental modes selected by arbitrary incidence conditions are evenly divided into two types. The types are distinguished by the sign of their absorption coefficient and by the sign of their energy flux through a plane of constant absorption. In a bounded crystal, they represent reflected and refracted beams. It is noteworthy that these results apply for arbitrary n, even though the solution of the n-beam equations only satisfies Maxwell's equations in the limit of infinite n. In this limit, the energy-conservation relation is equivalent to Poynting's theorem.

Introduction

It is known that the fundamental modes in the dynamical theory of X-ray diffraction may represent reflected as well as refracted beams. However, because the index of refraction for X-rays is nearly 1, reflected beams are usually negligible. An exception is two-beam Bragg diffraction from a thin-crystal plate (Zachariasen, 1945). For each state of polarization, a reflected and a refracted mode are excited. If the plate is sufficiently thin, interference between the two beams strongly modulates the rocking curve. As the thickness increases, the modulation rapidly disappears because the refracted beam is strongly damped by extinction and absorption before it is reflected from the exit surface. The absorption coefficients of

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