PENNINGTON, W. T., CHAKRABORTY, S., PAUL, I. C. & CURTIN, D. Y. (1988). *J. Am. Chem. Soc.* **110**, 6498–6504.
ROGERS, D. (1981). *Acta Cryst.* A**37**, 734–741.
SCHEFFER, J. R., TROTTER, J., GARCIA-GARIBAY, M. & WIREKO, F. C. (1988). *Mol. Cryst. Liq. Cryst. Incl. Nonlinear Opt.* **156**, 63–84.
TROTTER, J. & WIREKO, F. C. (1990). *Acta Cryst.* C**46**, 103–106.
ZIMMERMAN, H. E. (1980). *Molecular Rearrangements in Ground and Excited States*, edited by P. DE MAYO, ch. 16. New York: Wiley–Interscience.
ZIMMERMAN, H. E., KECK, G. E. & PFLEDERER, J. L. (1976). *J. Am. Chem. Soc.* **98**, 5574–5581.

Acta Cryst. (1990). B**46**, 440–446

The Use of Single-Wavelength Anomalous Scattering to Solve the Crystal Structure of a Gramicidin A/Caesium Chloride Complex

BY B. A. WALLACE*

Department of Chemistry and Center for Biophysics, Rensselaer Polytechnic Institute, Troy, NY 12180, *USA*

W. A. HENDRICKSON

Howard Hughes Medical Institute, Department of Biochemistry and Molecular Biophysics, Columbia University, New York, New York 10032, *USA, and Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, DC* 20375, *USA*

AND K. RAVIKUMAR

Department of Chemistry and Center for Biophysics, Rensselaer Polytechnic Institute, Troy, NY 12180, *USA*

(*Received* 12 *October* 1989; *accepted* 18 *January* 1990)

Abstract

Single-wavelength Cu $K\alpha$ anomalous scattering has been used to determine the structure of a crystalline complex of gramicidin A and caesium chloride. The asymmetric unit in these crystals, with space group $P2_12_12_1$ and $a = 32{\cdot}118\ (6)$, $b = 52{\cdot}103\ (12)$, $c = 31{\cdot}174\ (7)$ Å, contains four independent monomers (two dimers) of the pentadecapeptide. This structure falls in an intermediate size range for which direct methods and multiple isomorphous replacement are generally not successful for obtaining phase information. However, using the Bijvoet differences and the partial structure of the caesium atoms which have been incorporated in the crystals, it has been possible to obtain information on this crystal form. Because the caesium atoms dominate the scattering of these crystals, inclusion of the Friedel mate information in the restrained least-squares refinement has been essential. These studies extend the utility of single-wavelength anomalous-scattering phase determination to a macromolecular structure in which the partial structure of the anomalous scatterer is large.

Introduction

Gramicidin A is a linear pentadecapolypeptide antibiotic which forms dimeric channels in biological membranes; these channels are specific for the conductance of monovalent cations (Hladky & Haydon, 1972; Veatch & Stryer, 1977). Until recently (Wallace & Ravikumar, 1988; Langs, 1988), no crystal structure of gramicidin had been solved at the molecular level, because the molecule falls in a difficult size range for crystallographic studies: rather large for direct methods, and because of the difficulty in forming isomorphous derivatives of this flexible molecule, somewhat small for multiple isomorphous replacement phasing.

This paper describes the use of anomalous scattering from caesium atoms at a single wavelength (1·54 Å) far removed from the absorption edge of caesium for phase determination in crystals containing a complex of gramicidin and caesium chloride. This type of approach (Hendrickson, Smith & Sheriff, 1985), in which the phases are determined from a combination of the Bijvoet differences and the partial structure of the anomalous scatterer in a single crystal, has been used to solve the structures of a number of macromolecules, including crambin (Hendrickson & Teeter, 1981), myohemerythrin (Smith & Hendrickson, 1982) and trimeric hemerythrin (Smith, Hendrickson & Addison, 1983). In each of these cases, the anomalous scatterer was present in the native protein (the sulfur atoms of the cysteine side chains in crambin, and the iron centers in myohemerythrin and trimeric hemerythrin), and had

* Author to whom correspondence should be addressed.

0108-7681/90/030440-07$03.00

a relatively low calculated contribution to the total scattering. The present example differs because the anomalous signal was large and the partial structure of the added anomalous scatterer made a significant contribution to the total scattering of the crystal.

Structure determination

Crystallization

Gramicidin (ICN Nutritional Biochemicals), a mixture of 80% gramicidin A, 6% gramicidin B and 14% gramicidin C (variants with Phe and Tyr, respectively, at position 11) was recrystallized from absolute ethanol and then used without further purification. The gramicidin/CsCl complex was crystallized as previously described (Kimball & Wallace, 1984), except that the crystals used for data collection were prepared by addition of 190 μl of CsCl solution (100 m*M* in methanol) and 60 μl of methanol to 50 μl of gramicidin solution (150 mg ml^{-1} in methanol).

Data collection

Diffracted intensities were recorded on a Picker FACS-1 diffractometer at the Naval Research Laboratory, using the Vanderbilt program system (Lenhert, 1975). The crystal, of size 0·16 × 0·16 × 0·14 mm, was mounted in a capillary with *a* along the capillary axis. Intensity data to 1·8 Å resolution were collected using Ni-filtered Cu $K\alpha$ radiation. The peak-top ω scans were in seven steps of 5 s each in 0·03° intervals with a single 4 s background point. The integrated intensities were determined by a procedure based on the method of Hanson, Watenpaugh, Sieker & Jensen (1979) which fit the observed steps to Gaussians over a smoothly varying background. Friedel pairs at $(\varphi,\chi \pm 2\theta)$ were measured in blocks of 20 reflections to reduce systematic errors. The orientation matrix and the cell parameters were refined from the positions of 12 reflections. Radiation damage was monitored using two standard check reflections (one at 3·2 Å and one at 2·2 Å resolution) after every 200 reflections measured. At the end of the data collection, the 3·2 Å reflection had decayed to 77% of its initial value, while the 2·2 Å reflection had decreased to 57% of its initial value. Radiation damage corrections were made using data from two repeated sets of 4090 reflections ($d > 2{\cdot}5$ Å) collected at the beginning and end of the data set, and separated in time by 117 h. Reflections were sorted into 2θ shells containing equal numbers of reflections and the relative decay for each was fitted to a three-state model (Hendrickson, 1976) and used to correct the full data set. Intensities were further corrected for Lorentz and polarization effects and for absorption (North, Phillips & Mathews, 1968). Wilson statistics were used to determine the initial absolute scale factors (Wilson, 1949). Parameterized local scaling (Hendrickson & Teeter, 1981) was used to reduce residual systematic errors. Of the 10 354 measured reflections (including Friedel pairs), 9008 were above the 3σ significance level.

Caesium site determination

The Laue symmetry and the pattern of systematic absences in the diffraction patterns indicated the space group $P2_12_12_1$. However, due to the earlier suggestion of Koeppe, Hodgson, Berg & Stryer (1979) that crystals of a gramicidin/caesium thiocyanate complex with similar unit-cell dimensions and diffraction patterns for the $h0l$ and $0kl$ regions were of space group $P2_12_12_1$, it was decided to also test the possibility of that space group. Bijvoet-difference Patterson maps, calculated with coefficients of $(\Delta F)^2$ where $\Delta F = |F(h)| - |F(-h)|$ (Rossmann, 1961), were interpreted in the alternative space groups. Caesium positions were then refined and used to compute calculated Patterson maps for the best solutions in different space groups. The appearances of solutions in $P2_12_12_1$, $P2_12_12$, $P2_122_1$ and $P22_12_1$ were not very different; however, the $P2_12_12_1$ interpretation satisfied the features in the observed Patterson map more closely and gave lower *R* values as described below.

The best initial model in $P2_12_12_1$ had four caesium sites which were refined at full occupancy and with a thermal parameter $B = 7$ Å^2 to an *R* value of 0·350 at 2 Å resolution, by a full-matrix least-squares procedure based on *F*'s with unit weights (Hendrickson, 1985). A difference $(2F_o - F_c)$ map was then calculated, which revealed four additional sites, with electron densities corresponding to partially occupied caesium atoms. Upon inclusion of these additional sites (two each at constant occupancies of 0·65 and 0·40), the *R* value dropped to 0·322. Further refinement with a constant $B = 7$ Å^2 for all sites, and variable occupancies gave occupancies of 0·65, 0·64, 0·44 and 0·40 for these additional sites, while the occupancies of the original four sites remained near unity. The final *R* value based on a caesium model with eight sites was 0·308 at 2·0 Å resolution when refined against the 391 $|\Delta F|$ data for which $|\Delta F| \geq 2\sigma(\Delta F)$ and $|F(h)|$, $|F(-h)| \geq 3\sigma_F$. The standard *R* value for this model corresponds closely to the error *R* value of 0·305, obtained using $\sigma(\Delta F)$ in the numerator. The Patterson map calculated using this model (Fig. 1) showed excellent correspondence with the observed Patterson map. The locations of the eight caesium sites are indicated in Table 1. In anomalous-difference $(2F_o - F_c)$ maps, no other sites were apparent.

To confirm that the choice of space group was correct, the best four-site Patterson solution in $P2_12_12$ was also refined at full occupancy and with a $B = 7$ Å^2, giving an R value of 0·385 at 2 Å resolution. The inclusion of four extra sites improved this to $R = 0{\cdot}381$. As these R values were much higher than for the $P2_12_12_1$ solution, it seemed clear that the correct space group assignment was $P2_12_12_1$. Furthermore, it should be noted that assuming the four caesium sites suggested by Koeppe, Hodgson, Berg & Stryer (1979) for the gramicidin/caesium thiocyanate crystals, resulted in an R value of 0·401 at 2·0 Å resolution, and clearly was also not the correct solution for the caesium chloride crystals. This indicates a difference in the positions of at least the ions in the two crystal forms.

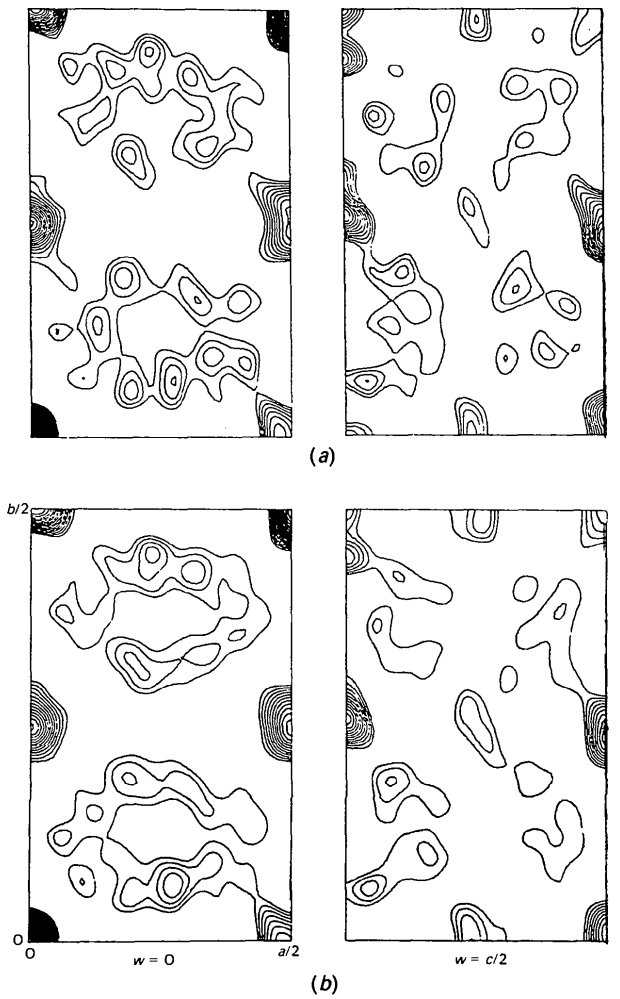

Fig. 1. (*a*) Observed and (*b*) calculated anomalous Patterson maps, calculated with $(\Delta F)^2$ coefficients. Reflections in the range 10·0–2·0 Å were included in the calculation, if they met the following criteria: $|F^+|, |F^-| \geq 3\sigma_F$; $|\Delta F| \geq 2\sigma(\Delta F)$.

Table 1. *Caesium sites determined from the anomalous Patterson solutions*

	x	*y*	*z*
Cs1	0·029 (2)	0·003 (1)	0·252 (2)
Cs2	0·061 (2)	0·233 (1)	0·273 (2)
Cs3	0·500 (2)	0·035 (1)	0·237 (2)
Cs4	0·473 (2)	0·257 (1)	0·258 (2)
Cs5	0·213 (3)	−0·041 (2)	0·221 (3)
Cs6	0·782 (3)	−0·027 (2)	0·280 (3)
Cs7	0·213 (3)	0·212 (3)	0·251 (4)
Cs8	0·776 (3)	0·223 (3)	0·269 (4)

Anomalous phasing

The calculated Bijvoet differences (Bijvoet, 1949) for eight caesium atoms (four partially occupied) and two gramicidin dimers per asymmetric unit was 17·8% $[\langle(\Delta F)^2\rangle/\langle|F_p|^2\rangle]$, with the partial structure of the caesium atoms estimated to be ~85% $(\langle|F_{As}|^2\rangle/\langle|F_p|^2\rangle)$, where F_{As} are the structure-factor magnitudes from anomalous scatterers. The measured Bijvoet difference ratio was 8·8% and the observed partial structure ratio was 43% for the caesium atoms.

Of the 4853 observed Friedel pairs of reflections to 1·8 Å, 894 were centrosymmetric. During data collection, differences in Friedel mates for general, but not centrosymmetric, reflections were readily apparent. One centrosymmetric reflection with $|\Delta F| >$ 3 r.m.s. (ΔF) was rejected as a distribution outlier. Using unimodal phases and combined probability phases from the caesium partial structure for the bimodal distributions (Hendrickson & Teeter, 1981), an F-weighted figure of merit of 0·72 for phases to 1·8 Å resolution was obtained.

The ambiguity as to the absolute configuration of the caesium structure remained to be resolved. Conventional single-wavelength anomalous phasing does not differentiate between enantiomers, but only the correct choice results in reasonable phases. Maps of both enantiomers were calculated and both resulted in cylindrical objects with a high density in the center. The task of making the choice of hand was made more difficult by the fact that gramicidin has alternating L and D amino acids and could, thus, form either left- for right-hand helices, so that, unlike typical all-L structures, the handedness of the backbone fold was not necessarily a clue. Upon careful examination of the maps, it could be seen that only one had connectivity that made chemical sense, and this map exhibited stronger internal symmetry. A least-squares fit, using the program *LSQRHO* (W. A. Hendrickson, unpublished results), was applied to a superposition of densities for the two dimers in the asymmetric unit. This gave a correlation coefficient of 0·78 for points within a 6 Å diameter cylinder and 0·74 for points within 8 Å. An intramolecular superposition about a diad perpen-

dicular to the cylinder axis gave a correlation coefficient of 0·54 for an 8 Å cylinder.

Pseudosymmetry considerations

The analysis of the crystal structure of gramicidin/CsCl was complicated somewhat by the pseudosymmetry relating the two dimers in each asymmetric unit. This pseudosymmetry is most strikingly evident in the virtual absence of ($h0l$) reflections having h odd or l odd for Bragg spacings greater than 4 Å. More generally, the intensities of reflections with $h + k = 2n$ (even) tend to be systematically stronger than those with $h + k = 2n + 1$ (odd), especially so at low scattering angles. Thus, the r.m.s. value of $|F(\text{odd})|$ is only 5·2% of the r.m.s. $|F(\text{even})|$ for those few reflections having $d > 10$ Å, but this fraction rises sharply to 30·0% in the 5–10 Å shell and it is up to 90·5% in the 2–2·5 Å shell $\{|F| = [|F(h)| + |F(-h)|]/2\}$. This pattern of intensities arises because each double-helical molecule is approximately aligned along a crystallographic screw axis – one at $(0,y,\frac{1}{4})$ and the other at $(\frac{1}{2},y,\frac{1}{4})$ – such that the two independent dimers are approximately related by a simple translation of $(\frac{1}{2},\frac{1}{2},0)$ with very little change in the azimuthal orientation.

The arrangement of major caesium sites on the molecular axes adds further to this pseudosymmetry. These sites are very near the centrosymmetric points of $(0,0,\frac{1}{4})$, $(0,\frac{1}{4},\frac{1}{4})$, $(\frac{1}{2},0,\frac{1}{4})$ and $(\frac{1}{2},\frac{1}{4},\frac{1}{4})$ and are thus nearly related by translations of $(\frac{1}{2},0,0)$ and $(0,\frac{1}{2},0)$. The partially occupied sites between molecules are in general positions, however. Consequently, the net contribution of the anomalous-scattering centers to the $h + k$ odd reflections is relatively greater than for the structure as a whole: the r.m.s. value of $|F_{Cs}(\text{odd})|$ is 39·5% of r.m.s. $|F_{Cs}(\text{even})|$ for $d >$ 10 Å, it rises to 55·6% in the 5–10 Å shell and it is at 90·0% in the 2–2·5 Å shell. The r.m.s. values of $|F_{Cs}(h = 2n,\ k = 2n)|$ relative to r.m.s. $|F_{Cs}(h = 2n + 1,\ k = 2n + 1)|$ are somewhat lower at 14·2, 30·0 and 69·5% for the respective shells, but even here the anomalous centers are relatively strong. Because of the partially pseudosymmetric configuration of caesium sites, the observed average magnitude of the Bijvoet differences is, as noted above, considerably less than expected from a random configuration. However, since contributions from the polypeptides themselves are also systematically weak for $h + k$ odd reflections, the anomalous phasing power is at least as strong for the odd as for the even reflections.

The approximately centrosymmetric character of the four major caesium sites has the implication that phases determined from the alternate enantiomer tend to be similar. Distinctions arise primarily from contributions of the four general, but partially occupied, intermolecular sites. It should be noted, however, that resolved anomalous phasing can be definitive even when based on centrosymmetric anomalous centers.

Model building and refinement

A detailed molecular model was constructed based on the 1·8 Å resolution electron density map. Map sections were calculated normal to the z axis, and the electron density was contoured at a scale of 5 mm Å^{-1} in sections separated by 0·8 Å. An averaged map was also calculated using the noncrystallographic symmetry described above. However, while the averaging improved the appearance of the backbone region of the molecule slightly, since the side-chain conformations are different in the different monomers, those regions were less well defined in the average map. Therefore, initial positional parameters for all (non-hydrogen) atoms of the polypeptide backbone and side chains were derived from the unaveraged minimap.

The pore-like structure of the polypeptide was immediately apparent from this map, and it was clear that two of the fully occupied caesium sites were located inside each of the two dimers that formed the asymmetric unit. The other partially occupied caesium sites were located between dimers. It was also found that there were three additional strongly scattering sites inside each pore, which could have been either chlorine or additional caesium sites. Inclusion of these sites as chlorine atoms with variable occupancies in the stereochemically restrained least-squares refinement (Hendrickson & Konnert, 1980) resulted in occupancies near unity, but this alone did not preclude them from being partially occupied caesium sites. However, if the sites were caesium atoms, their presence should have been observed in the earlier anomalous Patterson map. The absence of, for instance, peaks near $(0,\frac{1}{8},\frac{1}{2})$ and $(0,\frac{3}{8},\frac{1}{2})$, was consistent with the sites being chlorine atoms (since f'' for chlorine is less than 10% of f'' for caesium at the wavelength used). Hence, the new sites were assumed to be chlorine atoms.

Initial refinement was performed on data in the range of 5 to 3 Å resolution with the stereochemically restrained least-squares procedure, using averaged values for the Friedel mates, as was initially carried out with crambin (Hendrickson & Teeter, 1981) and myohemerythrin (Sheriff, Smith, & Hendrickson, 1987). A starting R value of 0·59 was obtained, and this was reduced to 0·38 after 17 cycles of refinement of positional and temperature factors. Data from 3 to 2 Å resolution were then included and after ten more cycles the refinement converged at $R = 0{\cdot}396$. The resulting model was used to calculate a $2F_o - F_c$ Fourier map. Model rebuilding was carried out using *FRODO* (Jones, 1978), followed by

more cycles of refinement. At this stage, the R value converged at 0·365 at 2·0 Å resolution. The caesium scattering seemed to contribute the majority of the error in phasing throughout these early stages of refinement, and small changes in the ion positions had dramatic effects on R.

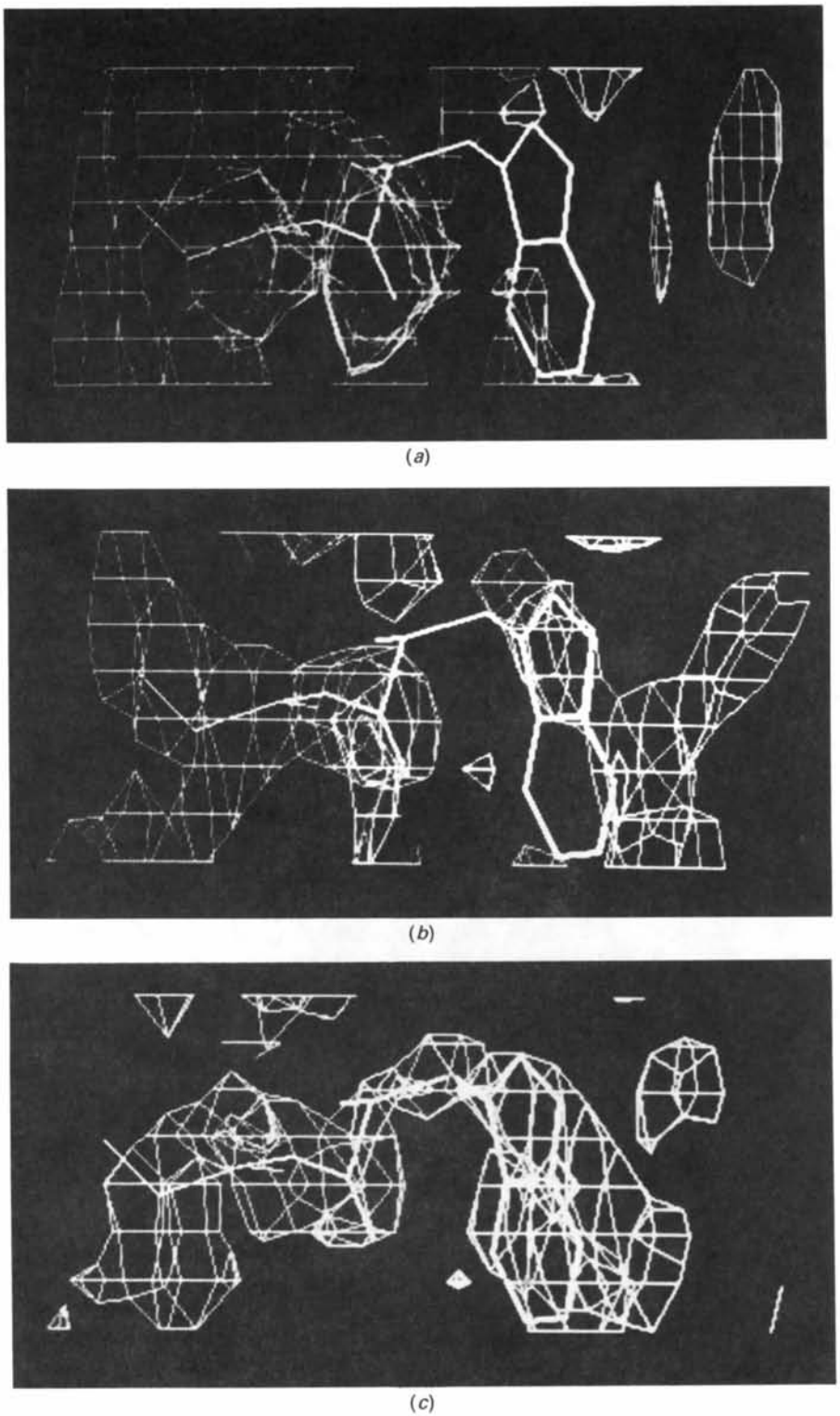

Fig. 2. Sections of the (*a*) initial electron density map, (*b*) the $(2F_o - F_c)$ map calculated using the average of the Friedel mates, and (*c*) the $(2F_o - F_c)$ map calculated using the individual $|F(h)|$ and $|F(-h)|$ terms. These show the relative fits of the side chain of Trp 13 of one of the monomers to the density.

The dominance of caesium scattering dictated that the individual $|F(h)|$ and $|F(-h)|$ terms should be included in the refinement at this point, with appropriate inclusion of anomalous-scattering contributions. It is interesting to note that the change to individual structure factors made little difference in the agreement factors for crambin and myohemerythrin, where the anomalous-scattering contributions are relatively small. However, in this case it had a major effect, and inclusion of individual terms was essential to the refinement: the R value dropped by 0·07 after 12 cycles and the quality of the $2F_o - F_c$ map improved remarkably (Fig. 2).

At first, manual rebuilding using the molecular graphics program *FRODO* (Jones, 1978) alternated between initial and $2F_o - F_c$ maps, but it was found that the initial phases were quite poor and changes made by following that map in preference to the difference map produced poorer results, so subsequent rebuilds concentrated on the difference maps. At the end, 38 solvent positions were determined from an $F_o - F_c$ map and were included in the refinement. The final R value to 2·0 Å resolution for reflections having $F \geq 3\sigma$ is 0·226. It is relatively constant across resolution shells, with the highest resolution shell having a value of 0·288. Removal of all the solvent molecules only increased the R value by about 0·07, thereby indicating that the solvent molecules (< 1 per residue) are not dominant contributors to the R-value calculation.

The resulting model is typified by an r.m.s deviation of 0·016 Å from ideal bond lengths. The average thermal parameter for all atoms is $B = 14{\cdot}7$ Å^2, with a range $6{\cdot}1 < B < 25{\cdot}7$ Å^2. The final refined caesium sites did not differ significantly from the refined caesium sites determined from the anomalous Patterson map.

Results and discussion

Gramicidin A has proved to be a significant challenge for crystallographic studies since crystals of this molecule were first prepared more than 40 years ago (Hodgkin, 1949). Using the method of single-wavelength anomalous scattering for phase determination, the structure of a caesium chloride complex of this membrane pore-forming molecule has now been determined.

In order to utilize this method, it was important to have crystals that diffracted to high Bragg angles and which contained a high caesium content. Koeppe, Hodgson & Stryer (1978) had reported a form of gramicidin/caesium crystals prepared from CsSCN, but these diffracted only to $d \sim 3$ Å. The gramicidin/CsSCN crystals grow large relatively rapidly, so part of our strategy was to slow down the rate of crystal formation in anticipation of producing fewer defects and better molecular ordering. Furthermore, a different, smaller counterion (Cl^-) was chosen as we thought that it might pack more readily in the channel. A higher caesium-to-gramicidin ratio was utilized (Kimball & Wallace, 1984) because circular dichroism spectroscopic monitoring of the caesium binding titration (Wallace, 1986; Callahan & Wallace, 1990) indicated that the ratios previously used to form crystals might not completely saturate

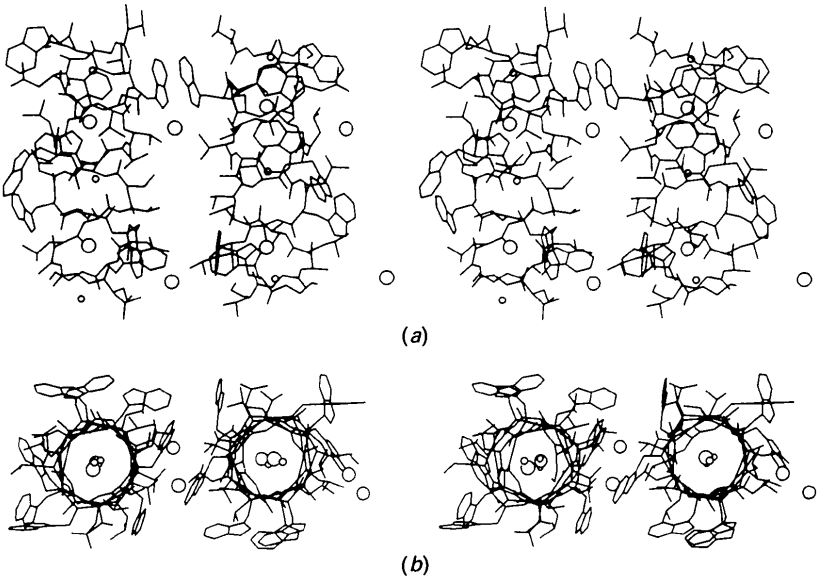

Fig. 3. Stereodiagrams of the gramicidin A/caesium chloride complex. View (*a*) is along the helix axes (crystallographic *b* axis), and view (*b*) is perpendicular to the helix axes (along the *c* axis). The two independent dimers in the asymmetric unit are shown. Caesium sites are indicated by large circles and chlorine sites by small circles.

the gramicidin binding sites, thereby resulting in a mixture of complexed and uncomplexed species in the crystallization solution.

The crystals used for these studies have eight caesium sites (four partially occupied) and two gramicidin dimers per asymmetric unit, so the calculated anomalous signal was 18%, with a partial structure of caesium of ~80%. Actual measured values were 8·8 and 43%, respectively, which was sufficient for initial phasing. That these values are lower than the calculated values appears to be a consequence of partial disorder in the caesium sites, and pseudocentering and symmetries which result in alternating near-zero intensity reflections.

Solution of the gramicidin/caesium chloride complex was not completely straightforward: not only was it difficult to obtain initial phases for this molecule, but initial model building was challenging because of the pseudosymmetries in the molecules and the relatively poor quality of the initial map. Refinement was hampered by the dominance of the caesium scattering and by the tight packing of molecules in the crystal. However, the data were sufficiently good to permit solution and refinement of the polypeptide, ions and a number of well-ordered solvent molecules. A full description of the molecular structure (Fig. 3) and a discussion of its biochemical implications is given elsewhere (Wallace & Ravikumar, 1988).

In summary, the studies described in this report demonstrate that the method of direct phasing using single-wavelength anomalous scattering is sufficiently powerful to solve this structure with an asymmetric unit of ~9000 daltons, and may ultimately prove important for solving other 'intermediate' size structures. The present work using a polypeptide/anomalous-scatterer complex extends the technique to a system in which the anomalous scatterer had a large partial structure, thus requiring different considerations for the refinement procedures.

We thank Krishna Murthy for helpful discussions regarding some of the refinement programs. This work was supported, in part, by NSF grants PCM82-15109, DMB87-96205 and DMB88-16981 (to BAW) and NIH grant GM34102 (to WAH).

References

BIJVOET, J. M. (1949). *Proc. Acad. Sci. Amst.* **B52**, 313–314.
CALLAHAN, J. D. & WALLACE, B. A. (1990). *Biochemistry*. Submitted.
HANSON, J. C., WATENPAUGH, K. D., SIEKER, L. & JENSEN, L. H. (1979). *Acta Cryst.* A**35**, 616–621.
HENDRICKSON, W. A. (1976). *J. Mol. Biol.* **106**, 889–893.
HENDRICKSON, W. A. (1985). In *Crystallographic Computing*, edited by G. M. SHELDRICK, C. KRUGER & R. GODDARD, Vol. 3, pp. 277–285. Oxford: Clarendon Press.
HENDRICKSON, W. A. & KONNERT, J. H. (1980). In *Computing in Crystallography*, edited by R. DIAMOND, S. RAMASASHAN & K. VENKATESAN, pp. 13.01–13.23. Bangalore: Indian Academy of Sciences.
HENDRICKSON, W. A., SMITH, J. L. & SHERIFF, S. (1985). *Methods Enzmol.* **115**, 41–55.
HENDRICKSON, W. A. & TEETER, M. M. (1981). *Nature (London)*, **290**, 107–113.
HLADKY, S. B. & HAYDON, D. A. (1972). *Biochim. Biophys. Acta*, **274**, 294–312.
HODGKIN, D. C. (1949). *Cold Spring Harbor Symp. Quant. Biol.* **14**, 65–78.
JONES, T. A. (1978). *J. Appl. Cryst.* **11**, 268–272.
KIMBALL, M. R. & WALLACE, B. A. (1984). *Ann. N. Y. Acad. Sci.* **435**, 551–554.
KOEPPE, R. E., HODGSON, K. O., BERG, J. M. & STRYER, L. (1979). *Nature (London)*, **279**, 723–725.
KOEPPE, R. E., HODGSON, K. O. & STRYER, L. (1978). *J. Mol. Biol.* **121**, 41–54.
LANGS, D. A. (1988). *Science*, **241**, 188–191.
LENHERT, P. J. (1975). *J. Appl. Cryst.* **8**, 568–570.
NORTH, A. C. T., PHILLIPS, D. C. & MATHEWS, F. S. (1968). *Acta Cryst.* A**24**, 351–359.
ROSSMANN, M. G. (1961). *Acta Cryst.* **14**, 383–388.
SHERIFF, S., SMITH, J. L. & HENDRICKSON, W. A. (1987). *J. Mol. Biol.* **197**, 273–296.
SMITH, J. L. & HENDRICKSON, W. A. (1982). In *Computational Crystallography*, edited by D. SAYRE, pp. 209–222. Oxford University Press.
SMITH, J. L., HENDRICKSON, W. A. & ADDISON, A. W. (1983). *Nature (London)*, **303**, 86–88.
VEATCH, W. R. & STRYER, L. (1977). *J. Mol. Biol.* **113**, 89–102.
WALLACE, B. A. (1986). *Biophys. J.* **49**, 295–306.
WALLACE, B. A. & RAVIKUMAR, K. (1988). *Science*, **241**, 182–187.
WILSON, A. J. C. (1949). *Acta Cryst.* **2**, 318–321.