Monoclinic uncomplexed double-stranded, antiparallel, left-handed $\beta^{5.6}$ -helix ($\uparrow\beta^{5.6}$) structure of gramicidin A: Alternate patterns of helical association and deformation

(crystal structure/ion channels/conformational transition)

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ABSTRACT A comparison of the monoclinic and orthorhombic crystal structures of the uncomplexed double-stranded, antiparallel, left-handed β -helix (5.6 amino acid residues per turn) ($\uparrow\beta^{5.6}$) conformers of gramicidin A reveals marked differences in the tryptophan side-chain orientations and the degree of helical uniformity of the dimer and in the manner in which these helical dimers associate with one another in the crystal. The helix of the orthorhombic dimer exhibits a regular pattern of bulges and constrictions that appears to be induced by crystal packing forces affecting tryptophan side chains that are aligned parallel to the helix axis. The monoclinic dimer is more uniform than the orthorhombic dimer as a consequence of π stacking interactions between dimers in which orientation of tryptophan side chains is normal to the helix axis to relieve the lateral crystal packing forces that may locally twist and deform the helix. It may be inferred from these observations that lipid interactions may be expected to destabilize the $\uparrow\beta^{5.6}$ helix when it is inserted into a membrane bilayer.

Cellular viability depends on an asymmetric distribution of ions—notably Na^+ , K^+ , Ca^{2+} , and Cl^- —across the membrane bilayer and on regulated ion-specific channels and pumps that can utilize and restore these gradients. Biological ion channels are either multidomain or multiple-subunit transmembrane proteins that span the lipid bilayer, although some primitive nonprotein channels have been characterized (1). Gramicidin A is a linear pentadecapeptide isolated from *Bacillus brevis* (2) that has an alternating D- and L-amino acid structural sequence, HCO-L-Val-D-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-L-Trp-D-Leu)₃-L-Trp-NHCH₂CH₂OH (3). Gramicidin A is primarily active against Gram-positive bacteria (4). It forms ion channels in bacterial membranes and in particular intracellular mitochondrial membranes, where influxes of cations such as K^+ activate excretory ATPases and kill the cell by depleting it of its ATP energy reserves (5). Gramicidin membrane channels are selective for monovalent cations of the alkali family as well as Ag^+ , Tl^+ , NH_4^+ , and H^+ (6–12). From spectra it is reported that the antibiotic exists predominantly as four different double-stranded dimeric conformations in organic solvents (13, 14), which upon absorption into lipid bilayers are transformed into an antiparallel head-to-head single-stranded $\beta^{6.3}$ dimer, that is reputed to be the major ion-conducting conformation of the antibiotic in membranes. A “zipper” mechanism has been proposed to suggest how these double-stranded dimers can be converted into single-stranded conducting channels within the membrane bilayer (15, 16). The crystal structure of the monoclinic

uncomplexed double-stranded, antiparallel, left-handed β -helix (5.6 amino acid residues per turn) ($\uparrow\beta^{5.6}$) form of gramicidin A crystallized from methanol is reported here and is compared with the conformation of the orthorhombic $\uparrow\beta^{5.6}$ dimer crystallized from ethanol (17).[§] Differences noted in the packing patterns and channel perturbations of the $\uparrow\beta^{5.6}$ helices in these two structures may have consequences with regard to the susceptibility of these dimers to transform into the single-stranded head-to-head $\beta^{6.3}$ form. The crystal structure of a CsCl complex of gramicidin A has been determined that has two independent ion-complexed dimers in the unit cell (18). Both molecular dimers are reported to have a hydrogen-bond pattern corresponding to an antiparallel, double-stranded, left-handed β -helix that has a $\uparrow\beta^{7.2}$ folding motif. The CsCl complex differs from the orthorhombic and monoclinic uncomplexed dimers in that the diameter of the helix is larger and it contains two Cs^+ and three Cl^- ions and an unspecified number of solvent sites within the pore.

MATERIALS AND METHODS

Data Measurement. Commercial grades of gramicidin A are mixtures of three isomers with an amino acid change at position 11: A ([Trp¹¹]gramicidin, $\approx 80\%$), B ([Phe¹¹]gramicidin, $\approx 6\%$), and C ([Tyr¹¹]gramicidin, $\approx 14\%$) isomers. A single crystal ($0.30 \times 0.20 \times 0.15$ mm) was grown from a saturated solution of methanol at 4°C and enclosed in a sealed glass capillary in the presence of a small amount of saturated mother liquor. X-ray intensity data were recorded on a Nonius-CAD4 diffractometer with graphite monochromated $\text{CuK}\alpha$ radiation at ambient room temperature (293 K) with an ω - 2θ scan procedure. Cell constants were refined by a least-squares fit of 25 carefully centered Bragg reflections in the $\sin\theta/\lambda$ range of 0.17 – $0.32 \text{ \AA}^{-1}$. A total of 4145 data were recorded to $1.5\text{-\AA}$ resolution of which 2973 were observed with $|F| > 2\sigma(|F|)$ after a Bayesian analysis (19) to improve the estimates of the weakly diffracting intensities. Unit cell data: monoclinic $P2_1$, $a = 15.155(9)$, $b = 26.48(1)$, $c = 32.13(1) \text{ \AA}$, $\beta = 92.03(5)^\circ$, $Z = 2$ for the dimer. These unit cell data are consistent with earlier values reported for gramicidin A crystallized from methanol (13, 20).

Structure Determination. Attempts to determine the crystal structure by direct phasing methods were hampered by the

Abbreviation: $\uparrow\beta^{5.6}$, double-stranded, antiparallel, left-handed β -helix with 5.6 amino acid residues per turn.

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[§]The refined atomic coordinates of the monoclinic uncomplexed form of $\uparrow\beta^{5.6}$ gramicidin A will be deposited and that of the orthorhombic uncomplexed form has been deposited with the Protein Data Bank and is available (ref. 1 GMA) in machine readable form from the Protein Data Bank at the Brookhaven National Laboratory or one of the affiliated centers at Melbourne or Osaka.

small number of significantly measured data and low probabilistic phasing weights for a structure of this complexity. Molecular replacement methods were equally unsuccessful in producing a well-defined orientation for the search model because of its helical symmetry (17, 21). The position of the helical axis was readily determined to be nearly parallel to the *c* axis of the crystal (22), but the maxima produced by the rotation function did not exhibit further significant variations as a function of the angular orientation of the helical search models with respect to this axis. Rotational searches utilizing the orthorhombic dimer, either with the side-chain conformations observed from its crystal structure or with the side chains terminated at the β -carbon positions on the helix, were unsuccessful. None of the many trial orientations that were examined led to a translation search from which the structure could be determined.

Eventually a trial model was selected that contained 193 of the 272 nonhydrogen atoms of the dimer. The side-chain positions that were incorporated included only those pairs of residues that had similar conformations on both peptide strands related by the approximate twofold axis of the dimer. This model included none of the tryptophan indole rings but most of the remaining valine and leucine side chains. A rotational search with this model using the 5.0- to 1.5-Å data produced three well-resolved maxima of equal height. One of these rotations produced four consistent, independent solutions for the T_1 , T_0 , Φ_1 , and Φ_0 translation functions (23) that ranked first, first, first, and second in magnitude in their corresponding maps. This molecular replacement solution had an initial *R* value of 0.53. However, all attempts to use tangent formula phase refinement methods, fragment recycling, and random phase extension to derive the missing side-chain positions for this model failed.

Structure Refinement. Inspection of the molecular replacement electron-density map on an Evans & Sutherland PS390 graphics system using FRODO (24, 25) revealed diffuse blobs of density near most of the missing tryptophan side-chain positions, but the orientations of the indole rings could not be modeled with confidence at this stage. It was decided to attempt to refine the replacement model to determine whether a more resolved density map would result. Fourteen cycles of constrained least-squares refinement were per-

formed using a VAX/VMS version of the Konnert and Hendrickson PROLSQ program (26, 27). During these 14 cycles of refinement, the resolution shells were slowly extended from 8.0–2.0 Å to 8.0–1.5 Å. The refinement residual converged from 0.53 to 0.465, and the atomic positions shifted an average rms displacement of 0.41 Å. Inspection of the following $2F_o - F_c$ map showed an improved definition of the electron density representing the missing tryptophan side chains (one of which even had a hole through the density that corresponded to the center of the six-membered ring of the indole). Two tryptophan side chains were added to the refinement model to increase the number of atoms to 213, and 10 subsequent cycles reduced the residual to 0.415. Several additional rounds of molecular graphics electron-density inspection, model building, and structural refinement were required to determine all of the remaining side-chain positions, during which the least-squares residual was reduced to 0.295.

Next we began to identify and refine the methanol solvent structure of the crystal. Of the 21 methanol molecules that were expected based on the density of the crystal, only 9 molecules were located that refined to meaningful contact geometries. Five of the alkyl side chains of the dimer appeared to exhibit some degree of discrete rotational disorder; these included Val-1, Val-7, and Leu-10 on chain 1 and Val-6 and Val-8 on chain 2 of the dimer. The site occupancies of these disordered groups were estimated from their relative densities in the $2F_o - F_c$ maps and were readjusted at various points in the refinement under the constraint that their paired occupation factors summed to 1.0. The refinement of this structure subsequently converged to a residual of 0.158 for 2942 data with $|F| > 2\sigma(|F|)$ between 8.0 and 1.5 Å resolution.

RESULTS AND DISCUSSION

Structure of the Dimer. The hydrogen-bonded pattern of the two peptide strands of the monoclinic gramicidin A dimer (Fig. 1) is that of a left-handed, antiparallel, double-stranded β -helix that has ≈ 5.6 amino acid residues per turn, which we designate $\beta^{5.6}$. The monoclinic $\beta^{5.6}$ dimer is nearly identical in conformation to the $\beta^{5.6}$ dimer structure that was determined for the orthorhombic crystal form obtained from

FIG. 1. Stereoview of the monoclinic form of the $\beta^{5.6}$ helix dimer of gramicidin A crystallized from methanol. The two strands of the dimer are differentiated by light and heavy lines, and only the major conformers of the disordered side-chain residues are illustrated. Note that the orientation of the indole rings of Trp-9 and Trp-11 on both strands is approximately normal to the helix axis.

FIG. 2. Stereoview of the orthorhombic form of the $11\beta^{5.6}$ helix dimer of gramicidin A obtained from ethanol. Only Trp-11 on the heavy chain is seen to be oriented approximately normal to the helix axis of the dimer.

ethanol solution (Fig. 2). The monoclinic channel is ≈ 31 Å long and has an average i.d. of 5.09 Å. Thus, it is the same length but appreciably larger in diameter than the orthorhombic channel form (4.80 Å).

Both channel forms contain bulges that appear to be large enough to accommodate water molecules, but these sites are not occupied in either structure. The orthorhombic channel contains three well-defined bulges > 5.25 Å that are separated by four channel constrictions < 4.5 Å, the smallest being 3.85 Å. When the constrictions at the two open ends of the monoclinic channel are ignored (4.25 and 4.50 Å), the narrowest diameter within the body of the channel is only 4.58 Å (between L-Ala-5 and L-Trp-13 on chains 1 and 2, respectively), while the largest channel bulge is 5.51 Å (between D-Leu-14 and L-Ala-5 on chains 1 and 2, respectively). All four constrictions within the orthorhombic channel are smaller than the tightest constriction within the monoclinic channel, and this value (4.58 Å) is thus not much smaller than the average diameter (4.80 Å) of the orthorhombic channel.

Table 1. ϕ and ψ torsion angles of the orthorhombic and monoclinic dimers

Residue	ϕ				ψ			
	O1	O2	M1	M2	O1	O2	M1	M2
Val-1	-135	-155	-136	-121	147	142	139	133
Gly-2	65	63	83	72	-141	-148	-151	-148
Ala-3	-151	-140	-138	-146	139	127	138	151
Leu-4	91	104	63	72	-140	-140	-132	-141
Ala-5	-160	-157	-164	-153	90	86	135	128
Val-6	108	119	97	99	-152	-158	-137	-141
Val-7	-139	-139	-143	-152	131	107	116	103
Val-8	89	119	92	105	-130	-146	-137	-143
Trp-9	-158	-159	-153	-159	105	98	121	117
Leu-10	104	102	101	113	-143	-135	-136	-141
Trp-11	-138	-155	-165	-163	113	122	106	98
Leu-12	98	98	120	117	-138	-131	-138	-130
Trp-13	-156	-161	-166	-165	82	89	97	117
Leu-14	138	120	125	107	-143	-141	-140	-143
Trp-15	-158	-157	-156	-151	81	97	99	90

Orthorhombic dimer chains 1 and 2 are designated by O1 and O2, while M1 and M2 refer to the monoclinic dimer strands.

The ϕ and ψ torsion angles of the monoclinic gramicidin A dimer are compared to those of the orthorhombic form in Table 1. The average values (and standard deviations from the mean) of the ϕ - ψ torsion angles of the D and L residues for the monoclinic structure are $\phi_L = -152(13)^\circ$, $\psi_L = 118(18)^\circ$, $\phi_D = 98(19)^\circ$, and $\psi_D = -140(6)^\circ$ as compared to $\phi_L = -153(9)^\circ$, $\psi_L = 110(22)^\circ$, $\phi_D = 101(20)^\circ$, and $\psi_D = -142(8)^\circ$ for the orthorhombic form. The closest agreement and smallest variances are associated with the ψ_D and ϕ_L values. The rms values for the unsigned, collective, pairwise differences in the ϕ and ψ values of the same positional amino acids related by the approximate twofold axis of the monoclinic and orthorhombic dimers are 4.8° and 7.7°, respectively, whereas the cross comparison between the monoclinic and orthorhombic channels leads to a slightly higher rms value of 9.2°. The corresponding pairwise rms deviation in the differences of the ψ_D torsion angles of the monoclinic dimer is only 2.4° as compared with $\approx 5^\circ$ for the other three torsional groups.

The majority of the valine and leucine residues of the monoclinic structure possess a side-chain conformation that is the most probable as reported in the literature (28), where 67% of the L-valine side chains have $\chi^1 = 173(9)^\circ$, and 64% of L-leucine side chains have $\chi^1 = -65(8)^\circ$ and $\chi^2 = 176(10)^\circ$. D residues are referred to the enantiomorph of these values, with C^{δ1}, C^{δ2} and C^γ, C^{γ2} interchanged to conform with the L configuration convention for labeling these atoms. The only fully ordered residue that does not adopt one of these most probable conformations is Val-7 on chain 2 ($\chi^1 = 63^\circ$, 5.4% probability). Among the discretely disordered residues, all have at least one partial occupancy in the most favored conformation, the only exception being Val-8 on chain 2, which has two conformations with 26.2% and 5.4% probability. The side-chain conformations reported for tryptophan from the literature are more variable, except that χ^2 has a strong preference for either $+90^\circ$ or -90° (28). A comparison of the tryptophan χ^1 and χ^2 torsion angles for the orthorhombic and monoclinic crystal structures is given in Table 2. With the exception of the two conformations of Trp-11 on chains 1 and 2 of the orthorhombic form ($\chi^1 = -65^\circ$ and 83° , respectively), the χ^1 values of the remaining 16 observations are tightly clustered and average $-168(13)^\circ$. The χ^2 values, in contrast, are seen to partition into two groups depending on whether the indole rings are normal

Table 2. Tryptophan side-chain conformation angles

	χ^1				χ^2			
	O1	O2	M1	M2	O1	O2	M1	M2
Trp-9	-182	-158	-170	-173	-114	-94	39	-7
Trp-11	-65	-178	-161	-161	-74	15	20	14
		83				-37		
Trp-13	-140	-166	-172	-194	-64	-90	-69	-121
		-183				80		
Trp-15	-165	-169	-154	-171	-108	-83	102	-100

χ^1 is the N-C α -C β -C γ torsion angle, and χ^2 is the C α -C β -C γ -C δ_1 torsion angle. Two entries appear for Trp-11 and Trp-13 under columns O2 to indicate that the side chains on strand 2 of the orthorhombic dimer display two discrete disordered orientations.

($\chi^2 \approx 0^\circ$) or parallel ($\chi^2 \approx -90^\circ$ or 90°) to the helix axis. Thus, in Table 2 there are 6 entries in the range -37° to 39° ($\chi^2 \approx 0^\circ$), 10 in the range -64° to -121° ($\chi^2 \approx -90^\circ$), and 2 entries having values of 80° and 102° ($\chi^2 \approx 90^\circ$).

Other β^5 structures that have been reported for alternating D-L polypeptides include Boc-(L-Val-D-Val) $_4$ -OCH $_3$ (Boc = *tert*-butoxycarbonyl; ref. 29), which forms a left-handed β^5 helix that is held together by 14 interstrand hydrogen bonds, and Boc-(L-Phe-D-Phe) $_4$ -OCH $_3$ (30), which in contrast forms a right-handed β^5 helix that requires only 12 interstrand hydrogen bonds. The averaged values of the helical torsion angles ϕ_L, ψ_L and ϕ_D, ψ_D for the left-handed valine peptide were reported to be $-138^\circ, 107^\circ$ and $109^\circ, -154^\circ$, respectively. Comparative values for a left-handed phenylalanine peptide can be inferred from its enantiomorph and are $-152^\circ, 113^\circ$ and $109^\circ, -154^\circ$, respectively. It may be noted that the ϕ_L average for the valine peptide (-138°) differs markedly from the values reported for the phenylalanine (-152°) and gramicidin ($-152^\circ, -153^\circ$) structures; and similarly, the ψ_D averages for the valine (-154°) and phenylalanine (-154°) peptides are markedly different than those observed for the gramicidin ($-140^\circ, -142^\circ$) structures. Thus, the interspecies torsional angle differences among these D,L peptides are considerably larger than the intraspecies differences between the monoclinic and orthorhombic forms of gramicidin, where the differences in torsion angles average only 3.5° .

Helical Packing Patterns. Differences between the orthorhombic and monoclinic channel forms extend to the manner in which each of these forms pack in the crystal. Channels of the orthorhombic form are aligned with the helix axis parallel to the 32-Å *b* axis of the crystal. In projection down the *b* axis, the channels form a hexagonal packing motif (Fig. 3). The side chains extending from consecutive turns of the helix overlap in this projection into six well-defined regions on the outer surface of the channel that accentuate the approximate sixfold symmetry of the helix. Because the helical repeat is actually closer to 5.75 residues per turn, the side-chain residues are aligned in ridges that twist slightly as they run the length of the channel. The helices pack in a hexagonal pattern that allows the protruding ridges of side chains on one helix to nest in the grooves formed between adjacent ridges on neighboring helices in the crystal. However, the channels did not pack in planar sheets but rather as alternating rows of parallel aligned helices displaced one-half of a unit cell in the *b* direction from neighboring rows in the crystal.

The channels of the monoclinic form are oriented with their helical axes parallel to the 31-Å *c* axis of the crystal, but in contrast with the orthorhombic form, these helices pack in coherent planar sheets with their symmetry-related neighbors. The packing within these sheets is approximately hexagonal (Fig. 4), as inferred from earlier studies (20), and involves a number of planar stacking interactions between the indole rings of Trp-9 and Trp-11 of both strands of the

FIG. 3. Crystallographic *b* axis projection illustrating the hexagonal packing of helix dimers for the orthorhombic structure. The lateral contact interactions between tryptophan side chains of neighboring helices are an important factor contributing to the bulges and constrictions observed in the diameter of the helix. Solvent ethanol molecules have been omitted for clarity.

dimer (Fig. 5). These stacking interactions orient the planes of the indole rings normal to the helical axis and permit the ridge lines populated by these tryptophan residues to interpenetrate as is shown by asterisks in Fig. 4. The lateral crystal packing forces involving Trp-9 and Trp-11 on either chain of the orthorhombic dimer may impose a deformation on the helix that does not exist in the monoclinic structure because of the ability of these planar stacked indole rings to slide past one another.

The crystal structure of the CsCl complex of gramicidin A that has been reported has two independent $\beta^{7.2}$ dimers in the structure (18). It is interesting to note that none of the 16

FIG. 4. The *c* axis projection of the monoclinic structure illustrating the intermediate square/hexagonal packing arrangement of $\beta^{5.6}$ helices in the crystal. Three distinct stacking interactions that allow the indole groups to slide laterally in the stack to relieve crystal packing forces are indicated by the labels A, B, and C and by asterisks in the figure. Solvent methanol molecules have been excluded for clarity.

FIG. 5. The *a* axis projection of the monoclinic structure further illustrating how the Trp-9 and Trp-11 indole rings stack within the helix sheet. The letters A, B, and C correspond to closest contact distances of 3.23, 3.35, and 3.24 Å, respectively. The labels on the tryptophan residues may be inferred from Fig. 1, which presents the same view.

indole planes in this structure appears to be oriented normal to the helix axis. Irregularities in the pore of the $\beta^{7.2}$ helix were observed, but these are primarily caused by differences in sizes of the Cs^+ , Cl^- , and solvent binding sites within the pore, rather than by external crystal packing forces, which are expected to be of secondary importance.

Liquid nitrogen temperature diffraction data have been recorded on the monoclinic gramicidin structure since this paper was originally submitted. Preliminary comparisons of the room and liquid nitrogen temperature structures indicate a significant ordering of the methanol solvent (16 full-occupancy and 5 half-occupancy sites) and a reduction in the thermal motion of functional side chains upon cooling. Val-1, Val-7, and Leu-10 on chain 1 become ordered, while Val-6 and Val-8 on chain 2 remain disordered. The Leu-10 side chain on chain 2, which was ordered at room temperature (B isotropic $\approx 16 \text{ Å}^2$), is seen to be disordered in the low-temperature study (B isotropic $\approx 6 \text{ Å}^2$). The conformation of the $\beta^{5.6}$ helix and the orientations of the tryptophan side chains are not noticeably perturbed in spite of the fact that the atomic coordinates of 267 atoms of the peptide, unaffected by disorder, undergo an rms displacement of 0.31 Å upon cooling. The bulk of this displacement is accounted for by a 0.15-Å shift of the dimer in the *xz* plane. The helix itself is not markedly deformed as the temperature is lowered, as could be the case if several of the tryptophan side chains shifted between horizontal and vertical planar orientations as a consequence of the solvent ordering.

A Raman study (31) of single-stranded $\beta^{6.3}$ dimers in liposomes has indicated a strong interaction between the lipid and the tryptophan side chains that forces all of the indole

rings into a parallel alignment with the helix axis to reduce the voids that would be introduced into the membrane bilayer if the orientation of these rings were normal to the helix axis. Assuming that other techniques may soon confirm this Raman evidence, it is reasonable to conclude that when the $\beta^{5.6}$ dimer is inserted into the membrane, the potential for tryptophan stacking interactions involving normal-aligned indole rings will be severely limited, and these rings will be forced into an orientation that is parallel to the helix axis. The lateral forces exerted by the lipid against the parallel-aligned tryptophan side chains could deform a uniform helical geometry, similar to what is seen in the monoclinic structure, to one possessing bulges and constrictions, as seen in the orthorhombic structure. Whether the deformations induced by these interactions are indicative of a helical destabilization mechanism whereby $\beta^{5.6}$ dimers interconvert to the ion-conducting single-stranded $\beta^{6.3}$ form remains to be demonstrated.

We dedicate this manuscript to the memory of our friend and colleague Prof. David Harker, recently deceased, who was one of the pioneers in the application of diffraction methods to determine molecular structure. We thank Dr. Jane Griffin for helpful suggestions concerning the preparation of this manuscript. Research support from National Institutes of Health Grants GM32812 and HL32303 is gratefully acknowledged.

1. Reusch, R. N. & Sadaff, H. L. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 4176–4180.
2. Dubois, R. J. (1939) *J. Exp. Med.* **70**, 1–10.
3. Sarges, R. & Witkop, B. (1965) *J. Am. Chem. Soc.* **87**, 2011–2027.
4. Hotchkiss, R. D. (1944) *Adv. Enzymol.* **4**, 153–199.
5. Harold, F. & Baarda, J. (1967) *J. Bacteriol.* **94**, 53–60.
6. Tosteson, D. C., Andreoli, T. E., Tiefenberg, M. & Cook, P. (1968) *J. Gen. Physiol.* **51**, 373–384 S.
7. Liberman, E. A. & Popaly, V. P. (1965) *Biochim. Biophys. Acta* **163**, 125–136.
8. Mueller, P. & Rudin, D. O. (1965) *Biochem. Biophys. Res. Commun.* **26**, 398–404.
9. Pressman, B. C. (1965) *Proc. Natl. Acad. Sci. USA* **53**, 1076–1083.
10. Eisenman, G., Sandblom, J. & Nehr, E. (1977) in *Metal-Ligand Interactions in Organic Chemistry and Biochemistry*, eds. Pullman, B. & Goldblum, B. (Reidel, Dordrecht, The Netherlands), Part 2, pp. 1–26.
11. Meyers, V. P. & Haydon, D. A. (1972) *Biochim. Biophys. Acta* **274**, 313–322.
12. Byrn, S. R. (1974) *Biochemistry* **13**, 5186–5193.
13. Veatch, W. R., Fossel, E. T. & Blout, E. R. (1974) *Biochemistry* **13**, 5249–5256.
14. Bystrov, V. F. & Arseniev, A. S. (1988) *Tetrahedron* **44**, 925–940.
15. Urry, D. W., Long, M. M., Jacobs, M. & Harris, R. D. (1975) *Ann. N.Y. Acad. Sci.* **264**, 203–220.
16. Killian, J. A. & De Kruijff, B. (1988) *Biophys. J.* **53**, 111–117.
17. Langs, D. A. (1988) *Science* **241**, 188–191.
18. Wallace, B. A. & Ravikumar, K. (1988) *Science* **241**, 182–187.
19. French, S. & Wilson, K. (1978) *Acta Crystallogr. A* **34**, 517–525.
20. Veatch, W. R. (1973) Ph.D. thesis (Harvard Univ., Cambridge, MA).
21. Nordman, C. E. (1972) *Acta Crystallogr. A* **28**, 134–143.
22. Koeppe, R. E., Hodgson, K. O. & Stryer, L. (1978) *J. Mol. Biol.* **121**, 41–54.
23. Langs, D. A. (1985) *Acta Crystallogr. A* **41**, 305–308.
24. Jones, T. A. (1985) *Methods Enzymol.* **115**, 157–171.
25. Pflugarth, J. W., Saper, M. A. & Quiocho, F. A. (1984) in *Methods and Applications in Crystallographic Computing*, eds. Hall, S. & Ashiaka, T. (Clarendon, Oxford), pp. 404–407.
26. Hendrickson, W. A. (1985) *Methods Enzymol.* **115**, 252–270.
27. Finzel, B. C. (1987) *J. Appl. Crystallogr.* **20**, 53–55.
28. Ponder, W. & Richards, F. M. (1987) *J. Mol. Biol.* **193**, 775–791.
29. Benedetti, E., Di Blasio, B., Pedone, C., Lorenzi, G. P., Tomasic, L. & Gramlich, V. (1979) *Nature (London)* **282**, 630.
30. Di Blasio, B., Benedetti, E., Pavone, V., Pedone, C., Gerber, C. & Lorenzi, G. P. (1988) *Biopolymers* **28**, 203–214.
31. Takeuchi, H., Nemoto, Y. & Harada, I. (1990) *Biochemistry* **29**, 1572–1579.