Lead Article

Acta Cryst. (1992). B48, 341-356

Folding, Aggregation and Molecular Recognition in Peptides

BY ISABELLA L. KARLE

Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, DC 20375-5000, *USA*

(*Received* 7 *October* 1991; *accepted* 14 *January* 1992)

Abstract

In past years, most of the X-ray structure determinations of oligopeptides were for cyclic peptides or for short linear peptides. Longer peptides usually presented many difficulties in obtaining suitable crystals since the molecules are intrinsically very flexible. More recently, it has been appreciated that the aminoisobutyric acid residue (Aib), which occurs naturally in many peptides of microbial origin, initiates helix folding. Several score of 7- to 15-residue linear peptides containing Aib have been synthesized, crystallized and had their structures determined to high resolution. Many of the peptide structures have been determined in more than one crystalline form. These peptide structures have yielded a plethora of information on types of helices, modes of hydration, water penetration into helical backbones, helices bent by Pro residues, parallel and antiparallel association of helices, effects of Leu residues on association of peptides, an example of a zipper assembly by side chains, and an example of a possible ion channel with a gating mechanism.

Introduction

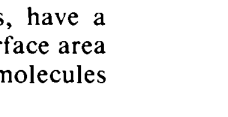
Chemical reactions usually involve interactions between molecules of unlike types. Molecular interactions of a different sort require a physical organization, such as a folding of a peptide or protein or a pre-assembly of like molecules. Examples include encapsulation of a cation by a peptide, molecular sieves formed by zeolites or clathrates, ion channels formed in membranes by peptides or the assembly of a virus from DNA components and a protein coat. Some examples demonstrate both self-assembly and assembly of unlike molecules. The assembly does not usually involve the formation of covalent bonds. Individual molecules may be held together by intra- or intermolecular hydrogen bonds, by hydrophobic interactions, by lock-and-key arrangements, double-helix or zipper mechanisms. Further, such assemblies may create cavities, channels or grooves that have the property of molecular recognition, that is, have a specific shape and/or polar or nonpolar surface area (inner or outer) that attract other kinds of molecules or that facilitate ion transport.

The main focus of this article will be on the aggregation motifs of helical peptides in crystals. Particular attention will be given to predominantly apolar peptides containing the Aib residue that occurs naturally in many peptides produced by microorganisms. The occurrence of both parallel and antiparallel helix aggregation will be demonstrated and the role of water molecules intimately associated with peptide molecules will be described. The stimulus for this structural study resulted initially from an interest in membrane-active peptides that encapsulate metallic ions (Pinkerton, Steinrauf & Dawkins, 1969), or that form voltage-sensitive ion channels (Mueller & Rudin, 1968; Fox & Richards, 1982; Nagaraj & Balaram, 1981). Subsequently, the design of four-helix bundles (Hecht, Richardson, Richardson & Ogden, 1990; Hill, Anderson, Wesson, De Grado & Eisenberg, 1990) from helical modules containing Aib residues (Karle, Flippen-Anderson, Sukumar, Uma & Balaram, 1991) and the demonstration of a zipper assembly (Landschulz, Johnson & McKnight, 1988) in the crystalline state (Karle, Flippen-Anderson, Uma & Balaram, 1990*a*) have been additional targets.

This article is intended to be illustrative, rather than an all-inclusive review of peptide structures. One of its purposes is to demonstrate the value of studying the structures of several pseudopolymorphs of particular peptides, by which means the conformational stability of a peptide can be assessed, the effect of the polarity of a solvent can be shown, hydration of an apolar peptide can be altered and, in some favorable cases, a static series (snapshots) encompassing a dynamic conformational change can be obtained. Almost all the crystal structures have been obtained to a resolution of ~0.9 Å with R values of 5 to 10%, allowing detailed features of conformation and hydrogen bonding to be observed with assurance.

Peptide characteristics

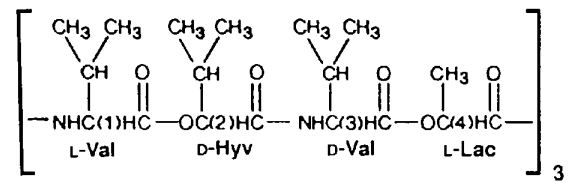
Peptides, like proteins, are composed of amino acid residues,



0108-7681/92/040341-16$06.00

where n can be as small as two or so large that the boundary between a peptide and a protein becomes indistinct. Natural peptides are produced by animal and plant sources, as well as by microorganisms. They function as hormones, regulators of bodily processes, ionophores, opiates, antibacterials and antimicrobials as well as potent toxins that are found in some mushrooms, blue-green algae, shellfish *etc.*

Peptides derived from saprophytic and parasitic plants or lowly marine organisms differ from proteins not only in size but also in composition and chirality of the amino acid residues. These peptides contain many residues with unusual side chains, in addition to the usual 20 residues found in proteins. Didemnin, isolated from a Caribbean tunicate (Hossain, van der Helm, Antel, Sheldrick, Sanduja & Weinheimer, 1988), is an extreme example of a peptide composed of unusual residues (Fig. 1). The –NH– moiety is sometimes replaced with an ether linkage –O– as, for example, in didemnin (above) and in valinomycin (Karle, 1975; Smith *et al.*, 1975; Karle & Flippen-Anderson, 1988) isolated from *Streptomyces fulvissimus*, in which case the compound is called a depsipeptide.



Furthermore, peptides like valinomycin and gramicidin A (Langs, 1988; Wallace & Ravikumar, 1988), isolated from *Bacillus brevis*, formyl-L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-ethanolamine, contain both D- and L-amino acids.

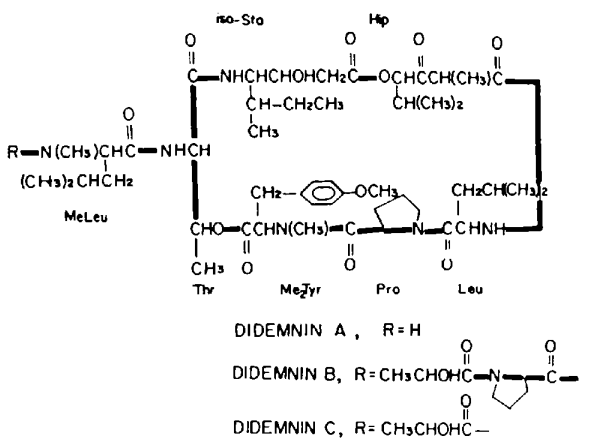


Fig. 1. Chemical structures of didemnins A, B and C (iso-Sta is isostatine) (Hossain, van der Helm, Antel, Sheldrick, Sanduja & Weinheimer 1988).

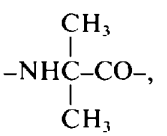
Backbones

The peptide backbone is not always linear as in proteins but can be cyclic. Crystal structures have been determined for cyclic peptides with 2 to 15 residues (Cook, Einspahr, Trapane, Urry & Bugg, 1980; Karle, 1981). Variations in cyclic backbones, as shown in Table 1 for selected naturally occurring peptides, can occur with linear peptides in which a portion has been cyclized by elimination of water between the carboxylic acid terminus and the OH in a threonine side chain or by a disulfide formation between two cysteine residues. Bridged cyclic peptides result when unusual side chains connect covalently across a cyclic backbone. Furthermore, peptides with cyclic backbones can complex with metal ions. Siderophores such as ferrichrome A (Zalkin, Forrester & Templeton, 1966) or ferrichrosin (Barnes, Eng-Wilmot & van der Helm, 1984) occur naturally with Fe^{3+} octahedrally ligated to O atoms in three side chains. Valinomycin forms a complex with K^+ octahedrally coordinated to six carbonyl O atoms that are part of the backbone (Neupert-Laves & Dobler, 1975) and antamanide complexes with Li^+ or Na^+ in a pentacoordinate fashion with four ligands to carbonyl O atoms in the backbone and the fifth ligand provided by a solvent molecule of various types (Karle, Karle, Wieland, Burgermeister, Faulstich & Witkop, 1973).

Linear peptides form β-sheets (Karle, Karle, Mastropaolo, Camerman & Camerman, 1983), 3_{10}-helices for short peptides (Toniolo *et al.*, 1983), α-helices for longer peptides (Karle & Balaram, 1990; Fox & Richards, 1982), double helices (Benedetti, di Blasio, Pedone, Lorenzi, Tomasic & Gramlich, 1979; Langs, 1988) and mixed helices consisting of a $3_{10}/\alpha$-helix and a β-ribbon twisted into a helix (Karle, Flippen-Anderson, Sukumar & Balaram, 1987; Karle, Flippen-Anderson, Agarwalla & Balaram, 1991). The latter has been established in Leu-zervamicin and a 16-residue apolar analog that contain in their sequences three Pro or Hyp (hydroxyproline) residues at positions 10, 13 and 15.

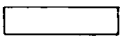
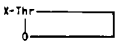
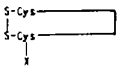
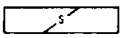
Helical peptides containing Aib residues

Linear peptides (8 to 20 residues) have generally been difficult to crystallize, presumably because of the large amount of flexibility inherent in the peptide backbone. The Aib residue,



has been shown both by calculating allowable conformational space (Marshall & Bosshard, 1972; Burgess & Leach, 1973; Prasad & Balaram, 1984) and experi-

Table 1. *Examples of types of backbones in naturally occurring peptides*

Type		Example	Reference
1. Cyclic		Antamanide	*A*
		Chlamydocin(dihydro)	*B*
	and/or	Cyclosporin	*C*
		Gramicidin S	*D*
		Valinomycin	*E*(1-3)
2. Cyclic plus linear		Didemnin	*F*
		Vernamycin	*G*
		Virginiamycin	*H*
		Oxytocin(deamino)	*I*
3. Bridged cyclic		β-Amanitin	*J*
4. Linear	β-Sheet	Enkephalin	*K*
	α-Helix	Alamethicin	*L*
	Double helix	Gramicidin A	*M, N*
	Bent, mixed helix	Zervamicin	*O*
5. Metal-ion encapsulation		Ferrichrome A	*P*
		Ferricrosin	*Q*
		K^+ valinomycin	*R*(1-2)
		$Na^+(Li^+)$antamanide	*S*

References: (*A*) Karle, Wieland, Schermer & Ottenheym (1979); (*B*) Flippen & Karle (1976); (*C*) Petcher, Weber & Ruegger (1976); (*D*) Hull, Karlsson, Main, Woolfson & Dodson (1978); (*E*1) Smith, Duax, Langs, De Titta, Edmonds, Rohrer & Weeks (1975); (*E*2) Karle (1975); (*E*3) Karle & Flippen-Anderson (1988); (*F*) Hossain, van der Helm, Antel, Sheldrick, Sanduja & Weinheimer (1988); (*G*) Karle & Flippen-Anderson (1990); (*H*) Declercq, Germain, Van Meerssche, Hull & Irwin (1978); (*I*) Wood, Tickle, Treharne, Pitts, Mascarenhas, Li, Husain, Cooper, Blundell, Hruby, Baku, Fischman & Wyssbrod (1986); (*J*) Kostansek, Lipscomb, Yocum & Thiessen (1978); (*K*) Karle, Karle, Mastropaolo, Camerman & Camerman (1983); (*L*) Fox & Richards (1982); (*M*) Langs (1988); (*N*) Wallace & Ravikumar (1988); (*O*) Karle, Flippen-Anderson, Agarwalla & Balaram (1991); (*P*) Zalkin, Forrester & Templeton (1966); (*Q*) Barnes, Eng-Wilmont & van der Helm (1984); (*R*1) Pinkerton, Steinrauf & Dawkins (1969); (*R*2) Neupert-Laves & Dobler (1975); (*S*) Karle (1985).

mentally (Fox & Richards, 1982; Toniolo *et al.*, 1983; Bosch, Jung, Schmitt & Winter, 1985) to limit severely the flexibility to an allowable region near $\varphi = \pm 57°$, $\psi = \pm 47°$ (where φ and ψ refer to rotations about the $N–C^\alpha$ and $C^\alpha–C'$ bonds, respectively) and to induce helix formation. Apparently to deal with the inherent flexibility of linear sequences in cases where structural rigidity is advantageous, several Aib residues have been incorporated naturally into peptide sequences such as in alamethicin, emerimicin, antiamoebin and zervamicin (Rinehart *et al.*, 1979) that produce voltage-gated ion channels in lipid membranes (Mueller & Rudin, 1968; Mathew & Balaram, 1983*a*). In recent years, a large number of peptides with 7 to 16 residues, all containing Aib, have been synthesized and crystallized and their structures have been established. The conformational and structural properties of 33 apolar helical peptides have been reviewed by Karle & Balaram (1990). Briefly, they all form helices, predominantly α-helices, with occasional 3_{10}-helix segments that occur mostly at the N terminus. The specific location of the Aib residues in the sequence is not very important for helix formation; for example, in at least one case an exchange of Aib with Leu produces no conformational changes (Karle, Flippen-Anderson, Uma & Balaram, 1990*b*), the elimination of Aib from the middle of a 16-residue sequence does not disturb the helix (Karle, Flippen-Anderson, Uma, Sukumar & Balaram, 1990) and the presence or absence of Aib at either end of the helix is immaterial (Karle, Flippen-Anderson, Uma & Balaram, 1990*c*). The number of Aib residues can be quite small. Nine-residue peptides containing only one Aib residue form a helix (unpublished). Peptides with ten or more residues and containing only one Aib residue have not been tested yet for helix formation.

Most of the apolar helical peptide crystals are grown by slow evaporation of solutions in methanol, ethanol, 2-propanol or ethylene glycol, with some water added. Often several crystalline forms for a particular peptide are produced. Although the packing in separate pseudopolymorphs may be quite different, and the kind and number of co-crystallized solvent molecules may be different, only rarely have there been more than minor changes in the conformation of the helical backbone.

Water associated with apolar helices

Head-to-tail region

Helical peptides form head-to-tail hydrogen bonds resulting in the formation of long rods of molecules in the crystal (Bosch, Jung, Schmitt & Winter, 1985; Francis, Vijayakumar, Balaram & Vijayan, 1985). The head-to-tail hydrogen bonding may consist of three

direct NH···O=C bonds in near-perfect register with the helical backbone, even for peptides with blocked termini, see an example in Fig. 8 (Karle, Flippen-Anderson, Uma, Balaram & Balaram, 1989; Karle, Flippen-Anderson, Sukumar & Balaram, 1992), or one or two direct NH···O=C bonds in combination with a mediating solvent molecule such as water or an alcohol, or hydrogen bonds involving side chains such as in Trp (Karle & Balaram, 1990). Often, especially in anhydrous crystals, the N(2)H or N(3)H moiety is bereft of any hydrogen-bonding possibilities.

Quite different water contents in the head-to-tail region can be observed in pseudopolymorphic crystals. The Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-(Val-Ala-Leu-Aib)$_2$-OMe peptide provides such an example (Karle, Flippen-Anderson, Uma, Sukumar & Balaram, 1990, 1992). The orthorhombic crystal obtained from CH_3OH/H_2O is stable only in contact with mother liquor, has six water sites in or near the head-to-tail region, as well as other water sites elsewhere, and very loose lateral packing between helices, Fig. 2(*a*), while the monoclinic crystal obtained from 2-propanol/H_2O has only two water sites, is stable dry, and has much more compact packing of helices (Fig. 2*b*), with a crystal density 8% higher than the crystal in Fig. 2(*a*). Despite the difference in water content and the packing, the conformation of the α-helix in both polymorphs is almost identical, the head-to-tail approach of the peptide molecules is the same and the hydrogen bonding for water molecules $W(1)$ and $W(2)$ is similar in both forms.

Apolar tripeptides are too short to form a turn in a helix. However, peptides such as Gly-Ala-Leu and Gly-Ala-Val, in effect, form one long continuous helix in the crystal by the mediation of two or three water molecules in the head-to-tail region. The water molecules enable the peptides to form a turn of the helix and to extend the helical structure through the crystal lattice (Ramasubbu & Parthasarathy, 1989; Parthasarathy, Chaturvedi & Go, 1990; Chaturvedi, Go & Parthasarathy, 1991).

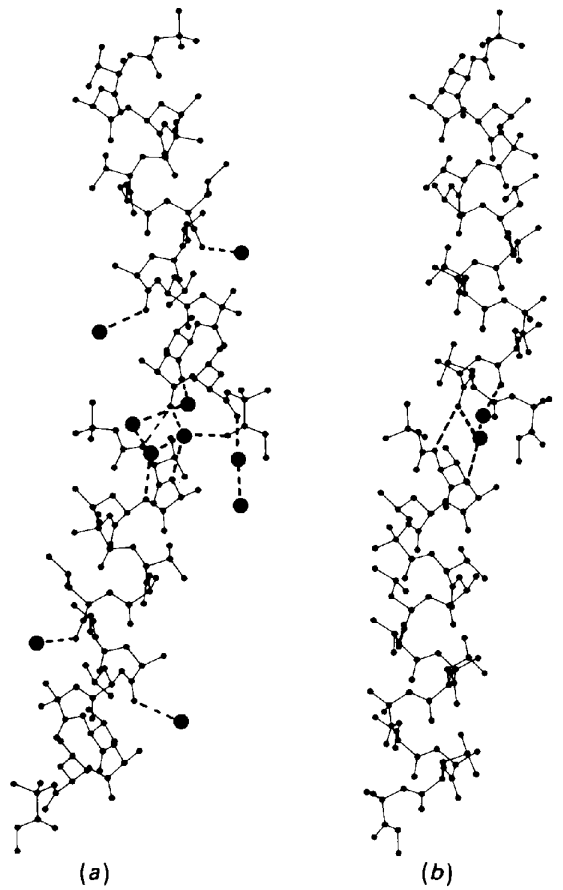


Fig. 2. Head-to-tail hydrogen bonding in the apolar helical peptide Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-(Val-Ala-Leu-Aib)$_2$-OMe in two different hydration forms. Water molecules are indicated by ●. Dashed lines indicate hydrogen bonds to water molecules and one direct N(1)···O(13) head-to-tail bond. (*a*) Crystal from CH_3OH/H_2O. (*b*) Crystal from 2-propanol/H_2O.

High-energy water

The term 'high-energy water' has been used for water molecules occurring in an unfavorable hydrophobic environment where the water cannot satiate its hydrogen-bond potential. Such water molecules are found in 'empty' cavities of supramolecules such as cyclodextrin (Saenger, 1980) and cyclophane (Smithrud & Diederich, 1990) among others (Schneider, Blatter & Zimmermann, 1990). In the packing of α-helical peptides composed of only apolar residues, hydrophobic cavities occur some-

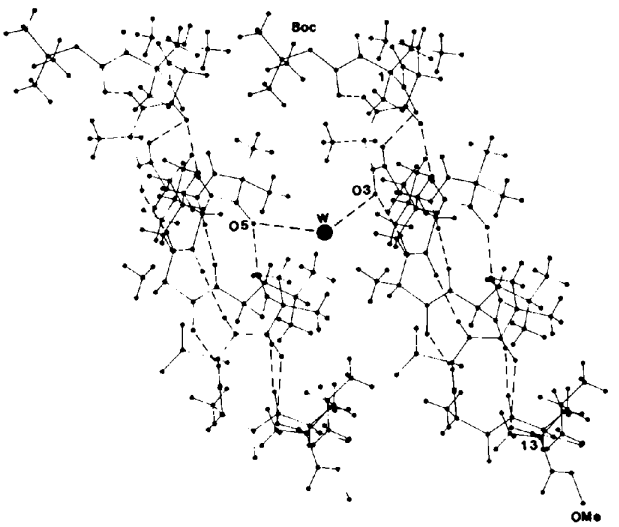


Fig. 3. Water molecule in hydrophobic cavity between helices of Boc-Aib-(Val-Ala-Leu-Aib)$_3$-OMe. The water molecule is a hydrogen-bond donor to carbonyl O(5) and carbonyl O(3) atoms in adjacent peptide molecules. These carbonyl O atoms are also involved in 5→1 hydrogen bonds in the α-helices (Karle, Flippen-Anderson, Uma & Balaram, 1989*a*).

times between helices. Water molecules have been found to occupy such cavities, as illustrated in Fig. 3 (Karle, Flippen-Anderson, Uma & Balaram, 1989*a*). In this case, the water molecule, although surrounded by $-CH_3$ groups from many side chains, is still able to form two hydrogen bonds with backbone carbonyl O atoms that also form NH···O=C bonds in the α-helix. In crystal structures of some other apolar helical peptides, the water molecules in hydrophobic cavities form only one hydrogen bond (Karle, Flippen-Anderson, Uma & Balaram, 1988*b*). Examples of water molecules in isolated hydrophobic holes that do not participate in any hydrogen bonding have not been documented in peptide structures.

Water penetration into helix backbones

Ideal apolar α-helices, as found in Boc-Aib-(Ala-Leu-Aib)$_3$-OMe (crystallized from ethylene glycol) (Karle, Flippen-Anderson, Uma & Balaram, 1992), become amphiphilic by the insertion of a water molecule into the backbone (crystal obtained from methanol/water) (Karle, Flippen-Anderson, Uma & Balaram, 1988*a*). Fig. 4 shows the breaking of an NH···O=C hydrogen bond in the helix and replacement by two hydrogen bonds between NH···O(*W*1) and C=O···HO(*W*1). In this case the resultant bending of the helix also exposes the carbonyl O(1) atom which attracts another water molecule *W*(2) to enhance the polar side of an originally apolar helix, rendering the peptide amphiphilic. Such mechanisms

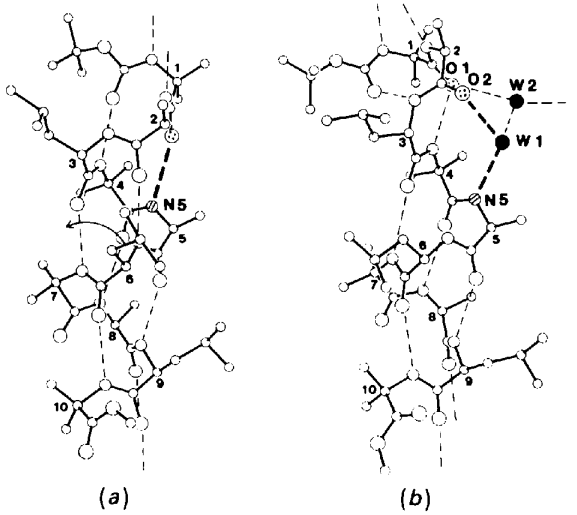


Fig. 4. Insertion of water into the α-helix of Boc-Aib-(Ala-Leu-Aib)$_3$-OMe that transforms an apolar helix into one with amphiphilic properties. (*a*) α-Helix obtained from ethylene glycol. (*b*) Hydrated helix obtained from methanol/water. Note the breaking of a helix hydrogen bond in (*a*) and the formation of two new hydrogen bonds to *W*1; the attraction of the additional *W*2; and the rotation of the Leu^6 side chain away from the new polar side of the helix (Karle, Flippen-Anderson, Uma & Balaram, 1988*a*).

may be involved in ion channels formed by helices with predominantly apolar residues, or they may be involved in the helix-folding or unfolding processes.

Observations of water insertions have also been made in the apolar structures of Boc-(Ala-Leu-Aib)$_2$-OMe and Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-OMe (Karle, Flippen-Anderson, Uma & Balaram, 1989*b*, 1990*c*). In the latter structure, both the unhydrated and the hydrated backbones occur side by side in the same unit cell. Water insertion into α-helical segments of proteins, with polar as well as apolar residues, have been found in troponin C (Satyshur, Rao, Pyzalski, Drendel, Greaser & Sundaralingam, 1988) and examined in well refined protein structures deposited in the Brookhaven Protein Data Bank (Sundaralingam & Sekharudu, 1989).

Effect of Pro or Hyp residues

Although Pro residues are generally considered helix breakers (Chou & Fasman, 1974), because of the lack of an NH moiety for an NH···O=C hydrogen bond, there are α-helices that contain the Pro or Hyp residue somewhere in the middle; see *e.g.* glyceraldehyde phosphate dehydrogenase (residues 146–161) (Richardson, 1981), melittin (Terwilliger & Eisenberg, 1982) and Leu-zervamicin (Karle, Flippen-Anderson, Agarwalla & Balaram, 1991). The effect of the bulk of the pyrrolidine ring is to cause a bend of ~30° or more in the helix and to expose a carbonyl O atom at the $i-3$ or $i-4$ position to the outside environment. Such an exposed carbonyl O atom is in a good position to attract a water molecule, even to an apolar helix. The importance of exposed carbonyls and water molecules will be illustrated in a later section on ion channels.

Packing motifs of apolar helices

Parallel and antiparallel association

A distinction should be made between helices containing only apolar residues and those containing both polar and apolar residues. Polar residues, with their capability to form hydrogen bonds with adjacent helical peptides, have an additional force that can have a major influence upon aggregation motifs. Except for the top and bottom of an apolar helix, the surface of the helix of a peptide like X-Trp-Ile-Ala-Aib-Ile-Val-Aib-Leu-Aib-Pro-OCH$_3$, shown in Fig. 5(*a*), is covered with methyl groups. Thus all lateral contacts of the helix are hydrophobic. Furthermore, the surface of the helix is composed of bulges and grooves (Chothia, Levitt & Richardson, 1981). For the sequence above, the bulges are emphasized by the occurrence of Ile or Leu at intervals of $i+3$.

The envelope of the helix in Fig. 5(*a*) is used to depict schematically the various modes of association that have been observed in pseudopolymorphic

crystals of the above peptide grown from a variety of solvents (Karle, Sukumar & Balaram, 1986; Karle, Flippen-Anderson, Sukumar & Balaram, 1988, 1990). The orientation of the left molecule in Figs. 5(*b*)-(*e*) is the same as in Fig. 5(*a*). Parallel association as illustrated in Figs. 5(*b*) and 5(*c*) has been found in space group $P2_1$ (where the twofold screw axis is parallel to the helix axis) and space group $P1$ (where all the peptide helices are related only by translation). The difference in the two parallel modes of association is a vertical shift of the bulges to the next groove. The same peptide was also crystallized in a different crystalline form with antiparallel association of the helices, shown in Fig. 5(*d*) (where the twofold screw axis of space group $P2_1$ is horizontal) in which bulges and grooves fit equally well. A second motif for antiparallel association in space group $P2_1$, shown in Fig. 5(*e*) (where the twofold screw axis is directed into the plane of the paper) has not yet been found in a pseudopolymorph of the above peptide, but has occurred in other apolar peptides such as Boc-Aib-Ala-Leu-Ala-Aib-Aib-Leu-Ala-Leu-Aib-OMe (Karle, Flippen-Anderson, Uma & Balaram, 1990*b*). Another apolar peptide in which all helices are parallel in one crystalline form and associate in an antiparallel motif in another crystalline form is Boc-Aib-Ala-Leu-Ala-Leu-Aib-Leu-Ala-Leu-Aib-OMe (Karle, Flippen-Anderson, Uma & Balaram, 1990*d*).

In the above examples, no specific side chains attract each other. The dominating factor in the packing motifs is shape selection, such as a bulge fitting into a groove. In the various packing motifs there are about an equal number of nearest C···C van der Waals approaches between adjacent helices in the range of ~3.8-4.1 Å. The proposal that the large dipole moments of α-helices are mainly responsible for the observed antiparallel association of helices in proteins (Hol, Halie & Sander, 1981) does not appear to apply to helices with only apolar residues. Helical peptides containing several polar side chains will be described in a later section.

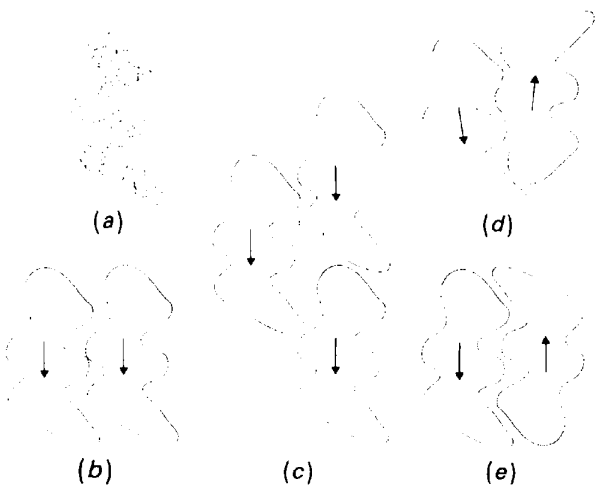


Fig. 5. Antiparallel and completely parallel packing motifs found for apolar helices. Shape selection, that is, bulge fitting into groove, and van der Waals attractions appear to be the dominating factors. (*a*) X-Trp-Ile-Ala-Aib-Ile-Val-Aib-Leu-Aib-Pro-OMe drawn with H atoms. (*b*) Envelope used to show all parallel packing in $P2_1$ with twofold screw axis nearly coincident with helix axis. (*c*) All parallel packing in $P1$. (*d*) Antiparallel packing in $P2_1$ with twofold screw horizontal. (*e*) Antiparallel packing with twofold screw axis directed into plane of paper [not found yet for peptide depicted in (*a*), but present in a number of other apolar decapeptides].

Elements of assembly

The assembly of helical molecules into a three-dimensional crystal can be described in three stages. First, there is a common element of helix assembly by the formation of long rods by head-to-tail hydrogen bonding (*vide supra*). There are two motifs for the helix columns, shown in Fig. 6(I): (*a*) the helices repeat from cell to cell with the helix axes aligned in a straight line or slightly tilted; and (*b*) the helices are related by a twofold screw, with the helix axes aligned in a straight line or zigzagged by a small amount. In the second stage, the columns associate into sheets with the various motifs shown in Fig. 6(II): (*a*)-(*c*) the columns in (I*a*) or (I*b*) are repeated by translation along a cell axis, roughly perpendicular to the rods; and (*d*) columns such as (I*a*) are related by a twofold screw axis located between and parallel to the columns. In the second stage, all helix axes are parallel (or approximately parallel), that is, all the N termini are pointing in the same direction. In the third stage, the sheets of parallel helices shown in Fig. 6(II) assemble into three-dimensional arrays as shown in Fig. 6(III): (*a*) crystals with completely parallel arrays of helices are formed by repetition of the sheets (as in space group $P1$) (Karle, Sukumar & Balaram, 1986) or a vertical twofold screw rotation of adjacent sheets (as one possibility in space group $P2_1$) (Karle, Flippen-Anderson, Sukumar & Balaram, 1988); (*b*) a crystal with antiparallel association of helices is formed by a rotation of adjacent sheets by a horizontal screw axis or a screw axis perpendicular to the page (Karle, Flippen-Anderson, Uma & Balaram, 1990*d*,*e*); (*c*) a skewed association of helices is obtained by an incomplete rotation of sheets about an axis perpendicular to the sheets (Karle, Flippen-Anderson, Uma & Balaram, 1990*b*). Peptides that have all parallel helix packing in one crystal form and antiparallel packing in another may have very similar arrangements in stages I and II, but have the sheets of parallel helices assemble differently in stage III (Karle, Flippen-Anderson, Sukumar & Balaram, 1990; Karle, Flippen-Anderson, Uma & Balaram, 1990*d*).

Examples of views looking into the helices are shown in Fig. 7 for various helix assemblies. A

hexagonal arrangement is common for antiparallel assemblies, as shown in Fig. 7(*a*) for Boc-Aib-Ala-Leu-Ala-Aib-Aib-Leu-Ala-Leu-Aib-OMe (Karle, Flippen-Anderson, Uma & Balaram, 1990*b*; see also Bosch, Jung, Schmitt & Winter, 1985; Okuyama, Tanaka, Doi & Narita, 1988; Francis, Iqbal, Balaram & Vijayan, 1983; Karle, Flippen-Anderson, Uma & Balaram, 1988*a*,*b*; Cerrini, Lamba, Scatturin, Rossi & Ughetto, 1989; Sugeta & Miyazawa, 1967). In this type of assembly, each helix has four antiparallel neighbors and two parallel neighbors. A checkerboard arrangement has also been found for antiparallel packing, for example, Fig. 7(*b*) in Boc-Aib-Leu-Aib-Aib-Leu-Leu-Leu-Aib-Leu-Aib-OMe

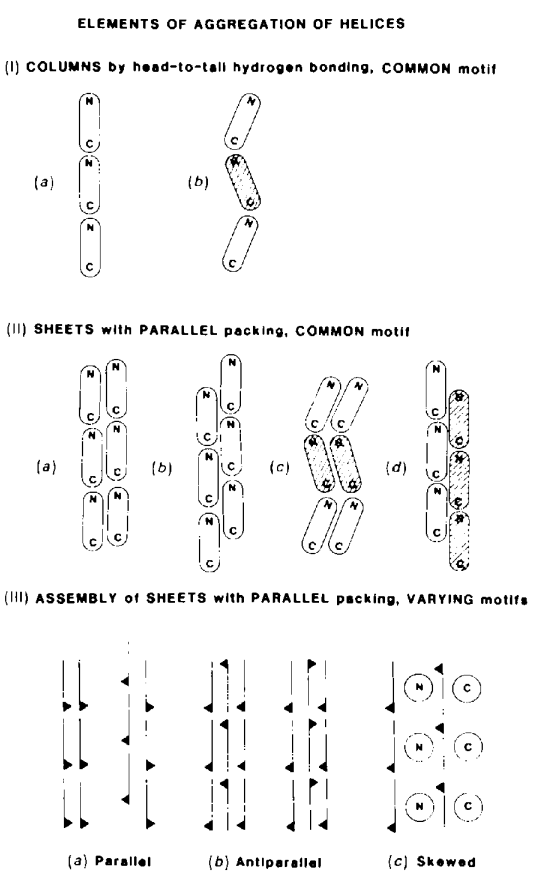


Fig. 6. Stages of helix aggregation. (I) Formation of rods or columns by head-to-tail hydrogen bonding: (*a*) by translation; (*b*) by a vertical twofold screw (the shaded diagram is the back of the helix as compared to the front in the unshaded diagram). (II) Assembly of rods into sheets. All motifs have parallel packing of helices, that is, all the N termini are pointed in the same (or nearly the same) direction. (III) Assembly of sheets (each set of three arrows aligned vertically represents an edge-on view of a sheet as shown in (II) into a three-dimensional array: (*a*) examples of two motifs for an all parallel assembly; (*b*) examples of two motifs for an antiparallel assembly; (*c*) a representative of a skewed assembly. The letters N and C indicate the N and C terminus, respectively, of individual helices. Examples of all of the above packing schemes have been found in crystal structures of apolar peptide helices.

(Karle, Flippen-Anderson, Sukumar & Balaram, 1991). In this case, each helix has four close antiparallel neighbors and four parallel neighbors at a greater distance. Assemblies with all helices parallel tend to lie on an approximately square grid, as shown in Fig. 7(*c*) for Boc-Aib-Ala-Leu-Ala-Leu-Aib-Leu-Ala-Leu-Aib-OMe (Karle, Flippen-Anderson, Uma & Balaram, 1990*d*). Sheets of peptides in a zigzag motif as in Fig. 6(II*c*), that undergo a rotation such as in Fig. 6(III*b*), will result in a skewed orientation of peptides lying in adjacent sheets, shown in Fig. 7(*d*). In this diagram, the middle sheet of peptides in three adjacent sheets has been rotated so that the view is directed into the helix of the center molecule of Boc-Aib-(Val-Ala-Leu-Aib)$_3$-OMe (Karle, Flippen-Anderon, Uma & Balaram, 1989*a*). Again, the fitting of a bulge containing a Leu side chain into a groove of an adjacent molecule between two Leu side chains is apparent. In Fig. 7(*e*), which illustrates pairs of helices with very long side chains that crystallize in a zipper motif (*vide infra*), the view from the top shows a herringbone pattern. The dimeric zippers pack parallel to each other along the long sides of the zipper, forming a thick parallel sheet. However, the thick sheets assemble in an antiparallel fashion (Karle, Flippen-Anderson, Uma & Balaram, 1990*a*).

Assemblies of a more complex nature have occurred in crystals of peptides with polar side chains, as in Ac-Glu-Leu-Leu-Lys-Lys-Leu-Leu-Glu-Glu-Leu-Lys-Gly-COOH (Hill, Anderson, Wesson, De Grado & Eisenberg, 1990).

Influence of side chains

Many of the apolar helical peptides whose crystal structures are available (Karle & Balaram, 1990) contain several Leu (or Ile) residues, affording a comparison of Leu···X attractions between helices. If the Leu or Ile residues are spaced by $i+3$ or $i+4$, they will occur on the same side of the helix and form bulges over each other. In Fig. 5, the bulges on the right side of the helix are formed by Ile^2, Ile^5 and Leu^8, those on the left by Aib^4 and Aib^7. As described earlier, the Ile (Leu) bulges fit between the Aib bulges in three different motifs with van der Waals contacts (~3.8–4.1 Å) between Leu and Aib. In Fig. 7(*d*), a leucyl bulge of one helix fits into a groove between leucyl side chains of a neighboring molecule that is skewed with respect to the first.

A commonly occurring motif is the Leu-Leu ladder shown in Fig. 8 for Boc-Aib-Leu-Aib-Aib-Leu-Leu-Leu-Aib-Leu-Aib-OMe (Karle, Flippen-Anderson, Sukumar & Balaram, 1992). In the diagram shown, the helices are parallel but shifted with respect to each other in the direction of the helix axis. In other ladder arrangements, the helices are at the same level, but they may be either parallel or antiparallel. In one

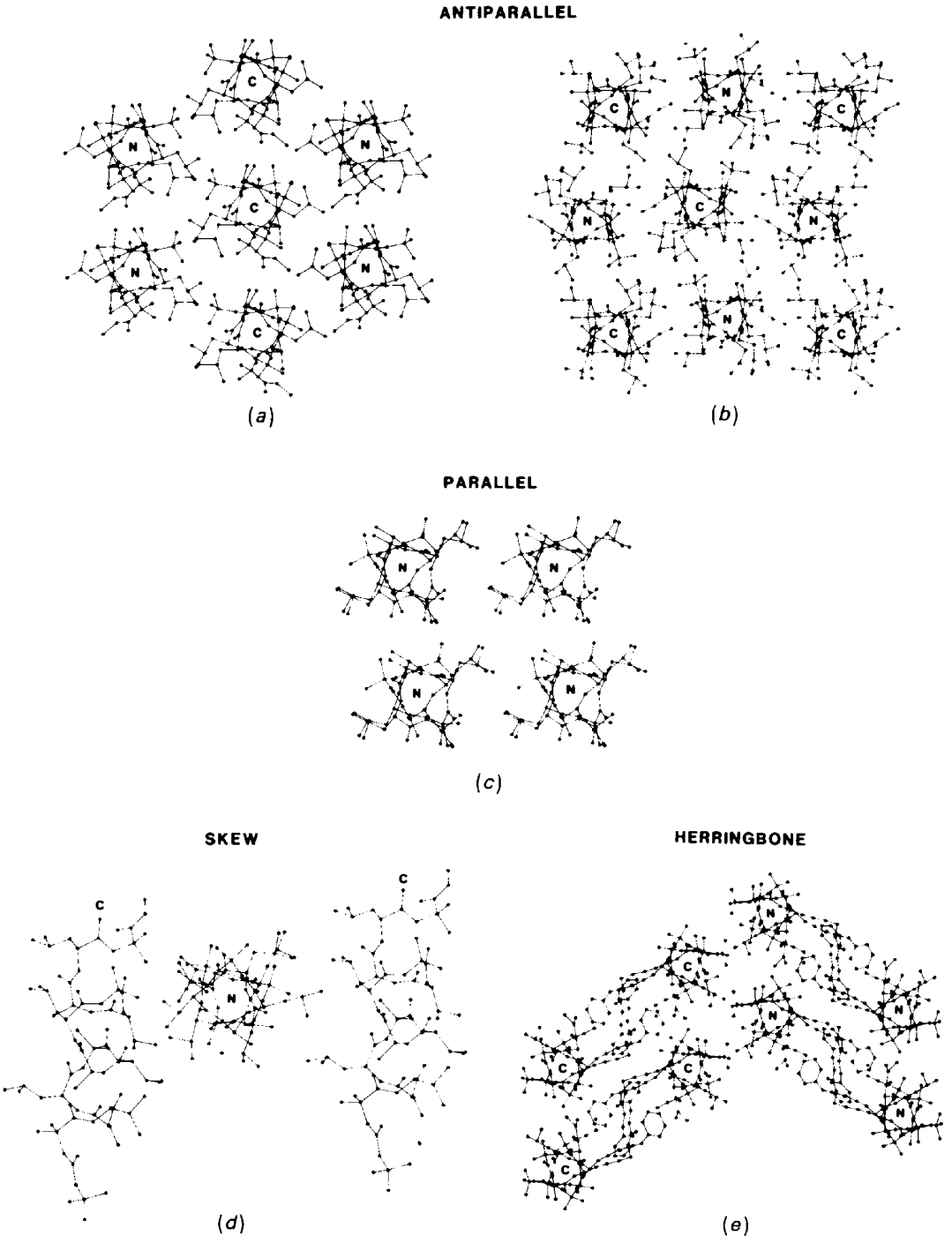


Fig. 7. Helix assemblies found in apolar peptides viewed down the helix axis. The top of each helix is labeled *N* for the N terminus and *C* for the C terminus. (*a*) Common pseudohexagonal grid for antiparallel packing. (*b*) Less common checkerboard grid for antiparallel packing. (*c*) Common nearly square grid for parallel packing. (*d*) Skewed packing of helices. (*e*) Packing for paired parallel helices in zippers.

crystalline form of Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-(Val-Ala-Leu-Aib)$_2$-OMe (Karle, Flippen-Anderson, Uma, Sukumar & Balaram, 1990), a parallel ladder is formed, but between Leu and Val residues.

In a number of structures the Leu residues do not show any particular tendency for association. Although orderly motifs are often recognized, the arrangement in any particular crystal is unpredictable. The variety of possible arrangements that are found in crystals involving only van der Waals contacts implies that they must be nearly isoenergetic.

Two-helix bundle

Four-helix bundles are a relatively common entity found in proteins, haemerythrin, for example. The design and synthesis of a four-helix bundle that would be stable and active without the remainder of the protein has been an active endeavor in recent years (Hecht, Richardson, Richardson & Ogden, 1990; De Grado, 1988). The propensity for α-helix formation in peptides containing the Aib residue has fostered attempts to design stable two-helix bundles as a preliminary step to four-helix bundles. The proposed procedure is to join two helices by a flexible linker segment that would result in a *U*-shaped molecule where the two helices would be adjacent to each other in an antiparallel mode. The crystal structure of Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-Acp-Val-Ala-Leu-Aib-Val-Ala-Leu-OMe, synthesized to demonstrate such a two-helix bundle, is shown in Fig. 9 (Karle, Flippen-Anderson, Sukumar, Uma & Balaram, 1991). The linker Acp is α-aminocaproic acid. Although the molecule has two seven-residue helical segments displaced with respect to each other and with their helix axes parallel, the linker has not folded the molecule. In the crystal, carbonyl O atoms at the bottom of the first helix make pseudo head-to-tail hydrogen bonds with the NH moieties at the top of the second helix in a neighboring molecule (one translation unit to the right or to the left), Fig. 10. The effect of the pseudo head-to-tail hydrogen bonding is to make long columns of helices in the crystal, but the columns

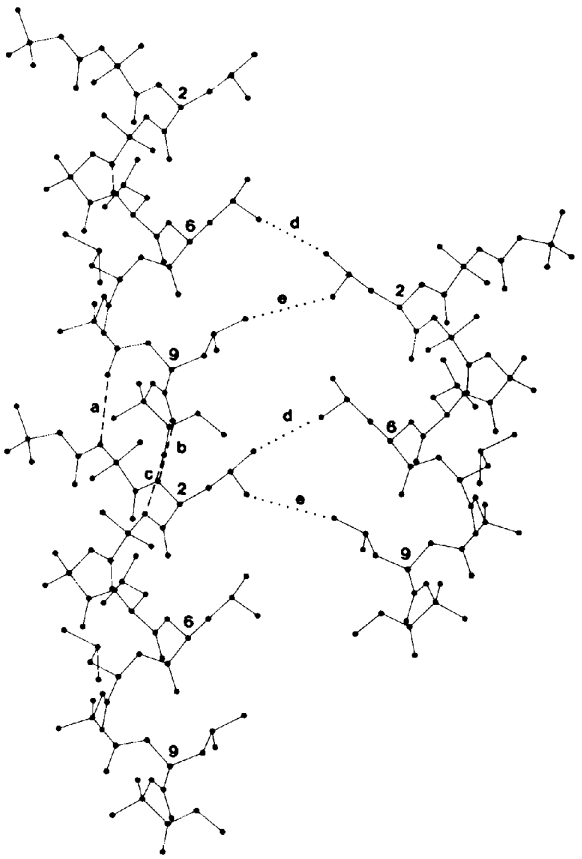


Fig. 8. Leu···Leu ladder motif between molecules of Boc-Aib-Leu-Aib-Aib-Leu-Leu-Leu-Aib-Leu-Aib-OMe. Adjacent helices, related by a twofold screw axis, are directed parallel to each other. The labels *a*, *b* and *c* refer to three head-to-tail hydrogen bonds in nearly perfect register with the helical backbones of the upper and lower molecules. The hydrogen bonds are N(1)···O(8) = 3.01, N(2)···O(9) = 2.89 and N(3)···O(10) = 3.06 Å, respectively. The labels *d* and *e* refer to van der Waals approaches between leucyl groups in neighboring molecules, with $C^{\delta}\cdots C^{\delta}$ distances of 3.88 and 3.93 Å, respectively (Karle, Flippen-Anderson, Sukumar & Balaram, 1992).

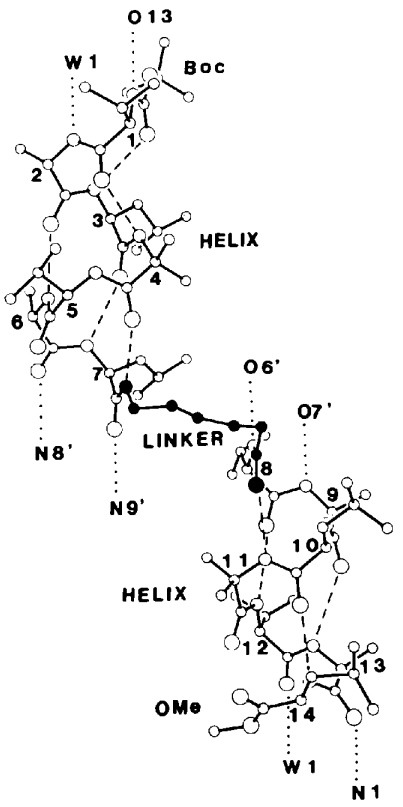


Fig. 9. Two helices joined by a linker. Head-to-tail and pseudo head-to-tail (in middle area) hydrogen bonds are indicated by dotted lines.

have alternating top and bottom helices from different lateral molecules. Control of spatial disposition of helices must require either a linker with a controlled conformation or specific favorable interactions between the helices. In the present case, the helices are composed of only apolar residues. Syntheses with other types of linkers are in progress.

Zipper motifs

A 'leucine zipper' has been hypothesized for helical segments of DNA-binding proteins in which every seventh residue is Leu (Landschulz, Johnson & McKnight, 1988; Vinson, Sigler & McKnight, 1989). The leucyl side chains from two adjacent helices were proposed to interdigitate in a zipper fashion. While this kind of leucine zipper has not been crystallographically characterized so far [the X-ray crystal structure of a peptide corresponding to the 'leucine zipper' segment of the yeast transcriptional activator GCN4 has shown it to be a parallel two-stranded coiled coil packed like the 'knobs into holes' model proposed by Crick (1953) (O'Shea, Klemm, Kim & Alber, 1991)], our model peptides have provided another kind of zipper arrangement. With the objective of introducing polar side chains oriented on the same helix face, Boc-Aib-Glu(OBzl)-Leu-Aib-Ala-Leu-Aib-Ala-Lys(Z)-Aib-OMe was synthesized. The peptide has two bulky apolar protecting groups on the functional side chains that are seven residues apart in the sequence. Intermolecular hydrogen bonds between extended Glu and Lys side chains enhance the head-to-tail hydrogen bonding (Fig. 11) (Karle, Flippen-Anderson, Uma & Balaram, 1990*a*). The peptide forms infinite columns with toothlike extensions of the Glu(OBzl) and Lys(Z) pairs on one side. The double teeth of one helix column interdigitate with the double teeth of another column, as shown in the stick model in Fig. 11 and the space-filling model in Fig. 12, to form a zipper assembly. Contacts between interdigitating side chains are entirely hydrophobic with van der Waals separations of 3.85-4.10 Å for C···C atoms.

Amphiphilic helices

Up to this point, the discussion has focused on helix association in peptides composed of apolar residues, the frequent lack of selectivity in lateral helix-helix interaction and the consequent multiple modes of assembly. Naturally occurring peptides usually contain at least several polar side chains in the sequence. Such polar side chains in helical peptides have a more distinct effect on helix association and certainly on the function of peptide. To date the structures of very few naturally occurring α-helical peptides have been published. Among them are: (*a*) melittin, NH_3-Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys-Arg-Lys-Arg-Gln-Gln-$CONH_2$, a 26-residue peptide found in bee venom, two molecules per asymmetric unit, 2.0 Å resolution, $R = 28\%$, structure solved by heavy-atom isomorphous derivatives (Terwilliger & Eisenberg, 1982); (*b*) alamethicin, Ac-Aib-Pro-Aib-Ala-Aib-Ala-Gln-Aib-Val-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Glu-Gln-Phol, a 20-residue peptide isolated from the soil fungus *Trichoderma viride*, three molecules per asymmetric unit, $R = 15.5\%$, structure solved by heavy-atom isomorphous derivatives, preliminary publication, coordinates are not available (Fox & Richards, 1982); (*c*) Leu-zervamicin, Ac-Leu-Ile-Gln-Iva-Ile-Thr-Aib-Leu-Aib-Hyp-Gln-Aib-Hyp-Aib-Pro-Phol, a 16-residue peptide isolated from the soil fungus *Emericellopsis salmosynnemata*, 0.93 Å resolution, $R = 10.1\%$, structure solved by stepwise direct phase determination of synthetic analog, then followed by search function (Karle, Flippen-Anderson, Agarwalla & Balaram, 1991); and (*d*) Asp-Thr-Ala-Ser-Asp-$(Ala)_6$-Leu-Thr-$(Ala)_2$-Asn-Ala-Lys-$(Ala)_3$-Glu-Leu-Thr-$(Ala)_2$-Asn-$(Ala)_7$-Thr-Ala-Arg, a 37-residue, completely α-helical, Ala-rich antifreeze peptide (AFP) from the winter flounder, two molecules per asymmetric unit, 2.5 Å resolution, $R = 27.4\%$, structure solved by analysis of Patterson map and modelling (Yang, Sax, Chakrabartty & Hew, 1988).

Helix characteristics

Melittin, alamethicin and Leu-zervamicin each fold into a helix (predominantly an α-helix) with a bend in the middle resulting in a banana shape. The bend occurs at Pro^{14} in melittin, Pro^{14} in alamethicin and Hyp^{10} in Leu-zervamicin. Usually a Pro residue is considered a helix breaker; however, in these peptides the Pro or Hyp residues are readily accommodated into a bend of the helix even though the N moiety cannot participate in hydrogen bonding. It is particularly remarkable that Leu-zervamicin has two Hyp residues and one Pro residue that are accommodated into a helical segment, although not an α-helix (Fig. 13). A further similarity among these three peptides is that the concave side of the bent helix is covered with the side chains of nonpolar residues, whereas the side chains of the hydrophilic residues are directed to the surface of the convex side. The polar convex side is enhanced further in each peptide by the exposed carbonyls that do not participate in backbone hydrogen bonding due to the presence of the Pro or Hyp residues; for example see the carbonyls of residues 6 and 7 in Fig. 13. The convex hydrophilic faces of these three peptides are accessible either to the solvent or for lateral hydrogen bonding to adjacent peptide molecules.

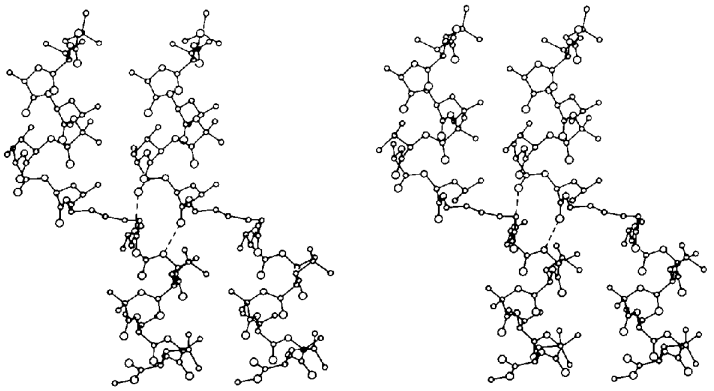


Fig. 10. Stereodiagram of association of two molecules of type shown in Fig. 9.

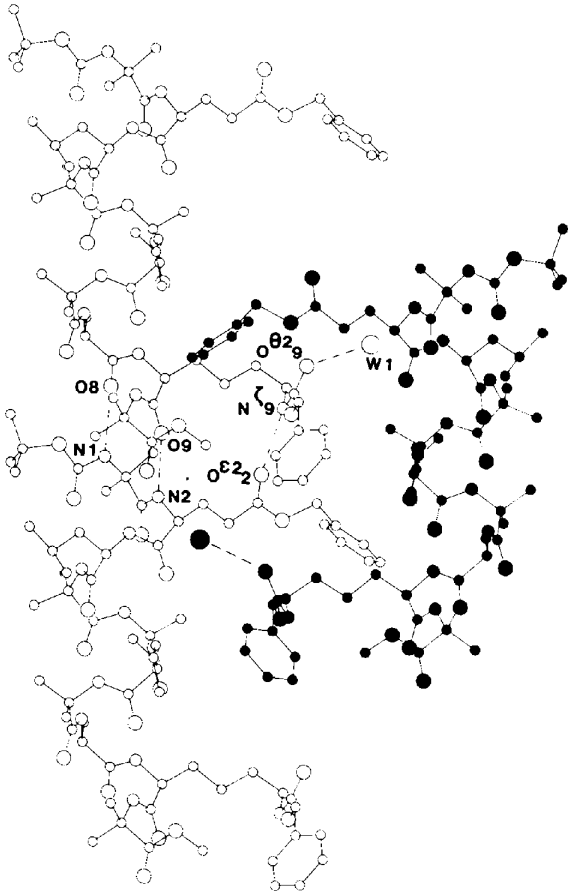


Fig. 11. Double-toothed zipper assembly in Boc-Aib-Glu(OBzl)-Leu-Aib-Ala-Leu-Aib-Ala-Lys(Z)-Aib-OMe. Two white molecules are shown with head-to-tail hydrogen bonding (dashed lines) that are a segment of a continuous column of repeating peptide molecules. The black molecule, representative of another continuous column related to the white column by a vertical twofold screw axis, does not have any hydrogen bonds in common with the white column. Interdigitation of side chains on residues 2 and 9 takes place by pairs, two white, two black *etc.*, in a zipper motif (Karle, Flippen-Anderson, Uma & Balaram, 1990*a*).

Fig. 12. Space-filling drawing of double-toothed zipper (courtesy of Jean M. Karle, Walter Reed Army Institute of Research). The view is in the same orientation as in Fig. 9.

Peptide assemblies

Four mellitin helices assemble into tetrameric units with very nearly 222 symmetry. The interior contacts of the assembly are almost completely between hydrophobic residues. On the other hand, the exterior of the tetramer contains the polar chains which are solvent accessible. Melittin appears to be surface active with respect to membranes and does not produce ion channels in the manner of alamethicin and zervamicin.

In the reported crystal structure of alamethicin, the three independent molecules form an array of irregular channels that are solvent filled. However, the channels are not lined with polar residues. In any single solvent channel both faces (hydrophobic and hydrophilic) contribute to the channel surface (Fox & Richards, 1982). Although the crystal structure did not give an image of a probable ion channel, the geometry of the alamethicin molecule has provided a great impetus for constructing model channels (Mathew & Balaram, 1983*b*; Hall, Vodyanoy, Balasubramanian & Marshall, 1984; Menestrina, Voges, Jung & Boheim, 1986).

Recently, four different crystal forms of Leu-zervamicin have been grown and analyzed. The two with the best resolution, 0.93 and 1.0 Å, have been refined to $R = 10.1$ and 12.1%, respectively, and most probably all the water molecules have been located. The other forms are in the process of least-squares refinement. In each crystal form the polar sides of the peptide molecules face each other in the same manner to form an interrupted water channel. However, the concave hydrophobic faces associate in an antiparallel motif in the $P2_1$ crystals and in a parallel motif in the $P2_12_12_1$ crystals (Karle, Flippen-Anderson, Agarwalla & Balaram, 1991). The interchangeability between parallel and antiparallel association has already been demonstrated for totally apolar helical peptides (*vide supra*).

Possible ion channel

The Leu-zervamicin molecule has a very similar conformation in the four polymorphic cells except for the degree of bending of the helix and the mobility of the Gln^3 side chain near the mouth. In the four polymorphs, the polar faces associate in the same antiparallel manner and form a water channel that is lined with polar groups. The channel is interrupted, however, by direct lateral hydrogen bonds between adjacent peptides, specifically $N^{\epsilon}H(Gln11)\cdots O{=}C(Thr6)$, $N^{\epsilon}H(Gln11)\cdots O^{\delta}(Hyp10)$ and $O^{\delta}H(Hyp10)\cdots O{=}C(Aib7)$ (Fig. 14). It appears to be significant that the long side chain of Gln11 folds away from the hydrophobic face of the molecule and wraps itself around the backbone in order to form hydrogen bonds on the hydrophilic side. Moreover, it can be hypothesized that the Gln11 side chain participates in a gating mechanism for opening and closing the channel. If the channel is opened under an applied potential (there is space for the Gln11 side chain to swing around in several different modes, even in the crystal), a single file of water molecules should be able to pass through. Thus, cations also must pass through the channel in a single file, pushing the column of water molecules along in front of them, as suggested for gramicidin channels (Stankovic, Heinemann & Schreiber, 1990).

At the mouth of the channel, the distance between N^{ϵ}(Gln3) and O(Phol16) varies by a large amount in the four polymorphs, 3.6 to >8 Å. The variation is caused by the different amount of bending in the helix (~30 to ~45°) and by the mobility of the side

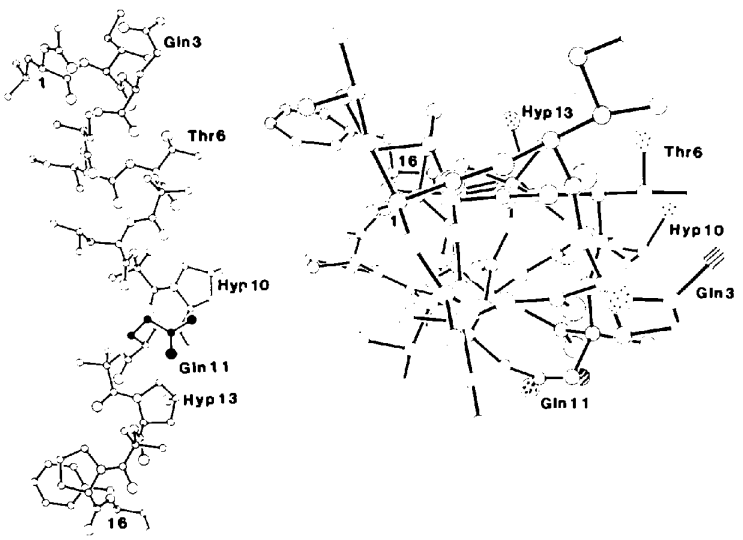


Fig. 13. Conformation of Leu^1-zervamicin with view perpendicular to curved helix (left) and into the helix (right). All the residues on the concave side are nonpolar and the polar residues are on the convex side. Note particularly the long side chain of Gln^{11} (darkened in left diagram) that wraps around the helix so that its polar $-C(O)NH_2$ end faces the polar face of the helix (Karle, Flippen-Anderson, Agarwalla & Balaram, 1991).

chain on Gln3 (folding toward its own helix or toward the helix on the other side of the water channel). The amount of water near the mouth of the interrupted water channels varies with the size of the mouth opening.

The structures of Leu-zervamicin appear to provide a glimpse of an ion channel and perhaps the importance of glutamine residues in which the side chains appear to be implicated in the gating mechanism for cation passage and in controlling the size of the mouth of the channel. In the crystal, the peptide helices repeat and the interrupted solvent channels repeat. It is not clear how many peptide helices are needed to form a channel in a membrane, whether only three helices are sufficient. Further, the channel is between antiparallel helices in the crystal. Does that mean that ion channels in membranes have antiparallel helices? Obviously more information, structural and otherwise, is needed. Diffraction experiments are continuing with antiamoebin,* another similar peptide ionophore.

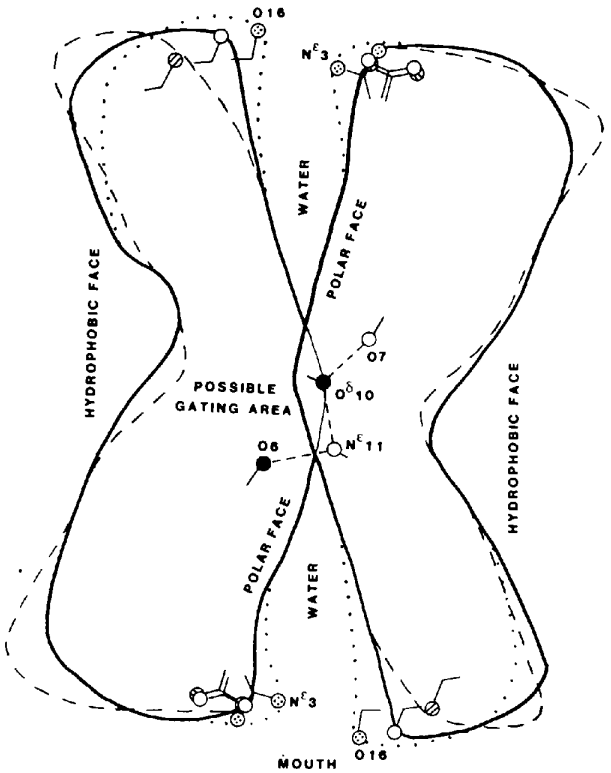


Fig. 14. Schematic diagram showing a superposition of the envelopes of Leu[1]-zervamicin in polymorph *A* (solid line), polymorph *B* (dotted line) and polymorph *C* (dashed line). The polar face of the helix associates in an antiparallel fashion and forms a water channel that is closed in the middle by *inter*peptide hydrogen bonds between side chains of Gln[11] and Hyp[10] and carbonyl O(6) and O(7) atoms. The number of water molecules varies with the bend of the helices and so does the size of the mouth of the channel.

Concluding remarks

Apolar helical peptides, primarily containing an α-helix for ten or more residues, are rather insensitive to modes of association. Aside from the common feature of head-to-tail hydrogen bonding that results in the long helical rods in the crystal, there are no other hydrogen bonds between apolar helical molecules. Laterally the helices associate in a crystal in a completely parallel fashion almost as often as in an antiparallel fashion. Skewed packing of helices has also been observed. The mode of packing is often recognized as a bulge in a groove type. The determining factor, which appears to be a maximum number of van der Waals approaches between neighboring molecules with C···C separations of 3.8–4.1 Å, is emphasized by the ease with which several crystalline pseudopolymorphs for a particular peptide can be obtained, often in a single crystallizing vial. No specific attractions have been observed for particular residues such as Leu. In some Leu-rich peptides, 'leucine ladders' are formed between neighbors, in others the side chains of Leu residues are not at all close to each other. In cases where cavities are formed between neighboring apolar peptides because of poor fits between hydrocarbon side chains, a water molecule may reside in the cavity if the cavity is large enough. Under such a circumstance the water molecules have been observed to participate in one, and sometimes two, hydrogen bonds with carbonyl groups that already have formed $5 \rightarrow 1$ type NH···OC bonds in the helical backbone.

Water is associated with apolar helical peptides more often than expected. In fact, water molecules can impart amphiphilic properties to apolar peptides. Aside from water molecules mediating head-to-tail hydrogen bonding in peptides where the NH and CO moieties do not meet in good register, and aside from water molecules filling cavities between helices, water molecules have been shown to insert into backbone helices (Karle, Flippen-Anderson, Uma & Balaram, 1988*a*; Sundaralingam & Sekharudu, 1989) by breaking a helix NH···OC bond and forming two new bonds, NH···*W* and *W*···OC. Under such a circumstance, the water molecule attracts either another water molecule or a different polar moiety and a polar surface is created on an apolar helix.

Minipolar areas are also created by Pro residues in the body of a helix. The bulk of the pyrrolidine ring causes a bend in the helix. Further, the carbonyl group that does not have a helix hydrogen bond, due to the presence of the Pro residue, extends to the outer environment and is free to attract water or possibly form ligands to metal ions. Many membrane active peptides contain a Pro or Hyp residue somewhere near the middle of the sequence (Rinehart *et al.*, 1979).

The structures of relatively few helical peptides with polar residues have been determined. Therefore, less-conclusive statements can be made concerning their structural and aggregational properties. Observations based on a very few examples are as follows. The bent helix found in Leu-zervamicin is almost identical to the one found in a 16-residue analog that had been stripped of the five polar moieties found in the Gln, Thr and Hyp residues in the natural peptide. In other words, these polar residues had no effect on the nature of the helix (Karle, Flippen-Anderson, Sukumar & Balaram, 1987; Karle, Flippen-Anderson, Agarwalla & Balaram, 1991). Further, lateral hydrogen bonds are formed between the polar side chains of neighboring molecules. In this case, the neighboring polar faces of the helices were antiparallel in all four polymorphs, while the neighboring nonpolar faces of the helices were either parallel or antiparallel.

X-ray diffraction analysis gives us an image of a static condition. A series of well defined static images can approximate a dynamic image. Often crystals of a substance are available before and after a change, and occasionally during a change, such as photo-rearrangements, metal-ion complexations, dimerizations, solvations, temperature and pressure variations. Work in progress on the four polymorphs of Leu-zervamicin will produce four static diagrams of the bent helix undergoing considerable changes in the channel and the size of the opening at the mouth. Other work in progress shows several stages of the unwinding of a helix with progressive solvation. Innumerable other questions concerning changes in peptide conformation or aggregation as a result of changes in the sequence or physical conditions, binding to ions or receptors and forming helix bundles, for example, are being actively addressed by X-ray diffraction methods in various laboratories.

This work was supported in part by the National Institutes of Health Grant GM30902 and the Office of Naval Research. The author wishes to acknowledge the indispensable collaboration of all her co-authors on peptide structures and especially Professor P. Balaram of the Indian Institute of Science in Bangalore for his insights, suggestions, syntheses and crystallizations of many of the peptides.

References

BARNES, C. L., ENG-WILMOT, D. L. & VAN DER HELM, D. (1984). *Acta Cryst.* C**40**, 922-926.

BENEDETTI, E., DI BLASIO, B., PEDONE, C., LORENZI, G. P., TOMASIC, L. & GRAMLICH, V. (1979). *Nature (London)*, **282**, 630.

BOSCH, R., JUNG, G., SCHMITT, H. & WINTER, W. (1985). *Biopolymers*, **24**, 961-978.

BURGESS, A. W. & LEACH, S. J. (1973). *Biopolymers*, **12**, 2599-2605.

CERRINI, S., LAMBA, D., SCATTURIN, A., ROSSI, C. & UGHETTO, G. (1989). *Biopolymers*, **28**, 409-420.

CHATURVEDI, S., GO, K. & PARTHASARATHY, R. (1991). *Biopolymers*, **31**, 397-407.

CHOTHIA, C., LEVITT, M. & RICHARDSON, D. (1981). *J. Mol. Biol.* **145**, 215-250.

CHOU, P. Y. & FASMAN, G. D. (1974). *Biochemistry*, **13**, 222-245.

COOK, W. J., EINSPAHR, H., TRAPANE, T. L., URRY, D. W. & BUGG, C. E. (1980). *J. Am. Chem. Soc.* **102**, 5502-5505.

CRICK, F. H. C. (1953). *Acta Cryst.* **6**, 639-689, 689-697.

DECLERCQ, J.-P., GERMAIN, G., VAN MEERSSCHE, M., HULL, S. E. & IRWIN, M. J. (1978). *Acta Cryst.* B**34**, 3644-3648.

DE GRADO, W. F. (1988). *Adv. Protein Chem.* **39**, 51-124.

FLIPPEN, J. L. & KARLE, I. L. (1976). *Biopolymers*, **15**, 1081-1092.

FOX, R. O. JR & RICHARDS, F. M. (1982). *Nature (London)*, **300**, 325-330.

FRANCIS, A. K., IQBAL, M., BALARAM, P. & VIJAYAN, M. (1983). *Biopolymers*, **22**, 1499-1505.

FRANCIS, A. K., VIJAYAKUMAR, E. K. S., BALARAM, P. & VIJAYAN, M. (1985). *Int. J. Pept. Protein Res.* **26**, 214-223.

HALL, J. E., VODYANOY, I., BALASUBRAMANIAN, T. M. & MARSHALL, G. R. (1984). *Biophys. J.* **45**, 233-247.

HECHT, M. H., RICHARDSON, J. S., RICHARDSON, D. C. & OGDEN, R. C. (1990). *Science*, **249**, 884-891.

HILL, C. P., ANDERSON, D. H., WESSON, L., DE GRADO, W. F. & EISENBERG, D. (1990). *Science*, **249**, 543-546.

HOL, W. G. J., HALIE, L. M. & SANDER, C. (1981). *Nature (London)*, **294**, 532-536.

HOSSAIN, M. B., VAN DER HELM, D., ANTEL, J., SHELDRICK, G. M., SANDUJA, S. K. & WEINHEIMER, A. J. (1988). *Proc. Natl Acad. Sci. USA*, **85**, 4118-4122.

HULL, S. E., KARLSSON, R., MAIN, P., WOOLFSON, M. M. & DODSON, E. J. (1978). *Nature (London)*, **275**, 207.

KARLE, I. L. (1975). *J. Am. Chem. Soc.* **97**, 4379-4386.

KARLE, I. L. (1981). In *The Peptides, Analysis, Synthesis, Biology*, Vol. 4, edited by E. GROSS & J. MEIENHOFER, pp. 1-54. New York: Academic Press.

KARLE, I. L. (1985). *Proc. Natl Acad. Sci. USA*, **82**, 7155-7159.

KARLE, I. L. & BALARAM, P. (1990). *Biochemistry*, **29**, 6747-6756.

KARLE, I. L. & FLIPPEN-ANDERSON, J. L. (1988). *J. Am. Chem. Soc.* **110**, 3253-3257.

KARLE, I. L. & FLIPPEN-ANDERSON, J. L. (1990). *Acta Cryst.* C**46**, 303-306.

KARLE, I. L., FLIPPEN-ANDERSON, J. L., AGARWALLA, S. & BALARAM, P. (1991). *Proc. Natl Acad. Sci. USA*, **88**, 5307-5311.

KARLE, I. L., FLIPPEN-ANDERSON, J. L., SUKUMAR, M. & BALARAM, P. (1987). *Proc. Natl Acad. Sci. USA*, **84**, 5087-5091.

KARLE, I. L., FLIPPEN-ANDERSON, J. L., SUKUMAR, M. & BALARAM, P. (1988). *Int. J. Pept. Protein Res.* **31**, 567-576.

KARLE, I. L., FLIPPEN-ANDERSON, J. L., SUKUMAR, M. & BALARAM, P. (1990). *Int. J. Pept. Protein Res.* **35**, 518-526.

KARLE, I. L., FLIPPEN-ANDERSON, J. L., SUKUMAR, M. & BALARAM, P. (1992). *Proteins Struct. Function Genet.* In the press.

KARLE, I. L., FLIPPEN-ANDERSON, J. L., SUKUMAR, M., UMA, K. & BALARAM, P. (1991). *J. Am. Chem. Soc.* **113**, 3952-3956.

KARLE, I. L., FLIPPEN-ANDERSON, J. L., UMA, K. & BALARAM, P. (1988*a*). *Proc. Natl Acad. Sci. USA*, **85**, 299-303.

KARLE, I. L., FLIPPEN-ANDERSON, J. L., UMA, K. & BALARAM, P. (1988*b*). *Int. J. Pept. Protein Res.* **32**, 536-543.

KARLE, I. L., FLIPPEN-ANDERSON, J. L., UMA, K. & BALARAM, P. (1989*a*). *Biochemistry*, **28**, 6696-6701.

KARLE, I. L., FLIPPEN-ANDERSON, J. L., UMA, K. & BALARAM, P. (1989*b*). *Biopolymers* **28**, 773-781.

KARLE, I. L., FLIPPEN-ANDERSON, J. L., UMA, K. & BALARAM, P. (1990*a*). *Proc. Natl Acad. Sci. USA*, **87**, 7921-7925.

KARLE, I. L., FLIPPEN-ANDERSON, J. L., UMA, K. & BALARAM, P. (1990*b*). *Biopolymers*, **30**, 719-731.

KARLE, I. L., FLIPPEN-ANDERSON, J. L., UMA, K. & BALARAM, P. (1990*c*). *Proteins Struct. Function Genet.* 7, 62-73.

KARLE, I. L., FLIPPEN-ANDERSON, J. L., UMA, K. & BALARAM, P. (1990*d*). *Biopolymers*, **29**, 1835-1845.

KARLE, I. L., FLIPPEN-ANDERSON, J. L., UMA, K. & BALARAM, P. (1990*e*). *Curr. Sci.* **59**, 875-885.
KARLE, I. L., FLIPPEN-ANDERSON, J. L., UMA, K. & BALARAM, P. (1992). In preparation.
KARLE, I. L., FLIPPEN-ANDERSON, J. L., UMA, K., BALARAM, H. & BALARAM, P. (1989). *Proc. Natl Acad. Sci. USA*, **86**, 765-769.
KARLE, I. L., FLIPPEN-ANDERSON, J. L., UMA, K., SUKUMAR, M. & BALARAM, P. (1990). *J. Am. Chem. Soc.* **112**, 9350-9356.
KARLE, I. L., FLIPPEN-ANDERSON, J. L., UMA, K., SUKUMAR, M. & BALARAM, P. (1992). In preparation.
KARLE, I. L., KARLE, J., MASTROPAOLO, D., CAMERMAN, A. & CAMERMAN, N. (1983). *Acta Cryst.* B**39**, 625-637.
KARLE, I. L., KARLE, J., WIELAND, T., BURGERMEISTER, W., FAULSTICH, H. & WITKOP, B. (1973). *Proc. Natl Acad. Sci. USA*, **70**, 1836-1840.
KARLE, I. L., SUKUMAR, M. & BALARAM, P. (1986). *Proc. Natl Acad. Sci. USA*, **83**, 9284-9288.
KARLE, I. L., WIELAND, T., SCHERMER, D. & OTTENHEYM, H. C. J. (1979). *Proc. Natl Acad. Sci. USA*, **76**, 1532-1536.
KOSTANSEK, E. C., LIPSCOMB, W. N., YOCUM, R. R. & THIESSEN, W. E. (1978). *Biochemistry*, **17**, 3790-3795.
LANDSCHULZ, W. H., JOHNSON, P. F. & MCKNIGHT, S. L. (1988). *Science*, **240**, 1759-1764.
LANGS, D. A. (1988). *Science*, **241**, 188-191.
MARSHALL, G. R. & BOSSHARD, H. E. (1972). *Circ. Res.* **30/31**, *Suppl.* II, 143-150.
MATHEW, M. K. & BALARAM, P. (1983*a*). *Mol. Cell. Biochem.* **50**, 47-64.
MATHEW, M. K. & BALARAM, P. (1983*b*). *FEBS Lett.* **157**, 1-5.
MENESTRINA, G., VOGES, K.-P., JUNG, G. & BOHEIM, G. (1986). *J. Membr. Biol.* **93**, 111-132.
MUELLER, P. & RUDIN, D. O. (1968). *Nature (London)*, **217**, 713-719.
NAGARAJ, R. & BALARAM, P. (1981). *Acc. Chem. Res.* **14**, 356-362.
NEUPERT-LAVES, K. & DOBLER, M. (1975). *Helv. Chim. Acta*, **58**, 432-442.
OKUYAMA, K., TANAKA, N., DOI, M. & NARITA, M. (1988). *Bull. Chem. Soc. Jpn*, **61**, 3115-3120.
O'SHEA, E. K., KLEMM, J. D., KIM, P. S. & ALBER, T. (1991). *Science*, **254**, 539-544.
PARTHASARATHY, R., CHATURVEDI, S. & GO, K. (1990). *Proc. Natl Acad. Sci. USA*, **87**, 871-875.
PETCHER, T. J., WEBER, H. P. & RUEGGER, A. (1976). *Helv. Chim. Acta*, **59**, 1480-1489.
PINKERTON, M., STEINRAUF, L. K. & DAWKINS, P. (1969). *Biophys. Res. Commun.* **35**, 512-518.
PRASAD, B. V. V. & BALARAM, P. (1984). *CRC Crit. Rev. Biochem.* **16**, 307-348.
RAMASUBBU, N. & PARTHASARATHY, R. (1989). *Biopolymers*, **31**, 397-407.
RICHARDSON, J. S. (1981). *Adv. Protein Struct.* **34**, 167-339.
RINEHART, K. L. JR, PANDEY, R. C., MOORE, M. L., TARBOX, S. R., SNELLING, C. R., COOK, J. C. JR & MILBERG, R. H. (1979). In *Peptides. Proc. 6th American Peptide Symposium*, edited by E. GROSS & J. MEIENHOFER, pp. 59-71. Rockford, IL: Pierce Chemical Co.
SAENGER, W. (1980). *Angew. Chem. Int. Ed. Engl.* **11**, 344-362.
SATYSHUR, K. A., RAO, S. T., PYZALSKA, D., DRENDEL, W., GREASER, M. & SUNDARALINGAM, M. (1988). *J. Biol. Chem.* **263**, 1628-1647.
SCHNEIDER, H.-J., BLATTER, T. & ZIMMERMAN, P. (1990). *Angew. Chem. Int. Ed. Engl.* **29**, 1161-1162.
SMITH, G. D., DUAX, W. L., LANGS, D. A., DE TITTA, G. T., EDMONDS, J. W., ROHRER, D. C. & WEEKS, C. M. (1975). *J. Am. Chem. Soc.* **97**, 7242-7247.
SMITHRUD, D. B. & DIEDERICH, F. (1990). *J. Am. Chem. Soc.* **112**, 339-343.
STANKOVIC, C. J., HEINEMANN, S. H. & SCHREIBER, S. L. (1990). *J. Am. Chem. Soc.* **112**, 3702-3704.
SUGETA, H. & MIYAZAWA, T. (1967). *Biopolymers*, **5**, 673-678.
SUNDARALINGAM, M. & SEKHARUDU, Y. C. (1989). *Science*, **244**, 1333-1337.
TERWILLIGER, T. C. & EISENBERG, D. (1982). *J. Biol. Chem.* **257**, 6016-6022.
TONIOLO, C., BONORA, G. M., BAVOSO, A., BENEDETTI, E., DI BLASIO, B., PAVONE, V. & PEDONE, C. (1983). *Biopolymers*, **22**, 205-215.
VINSON, C. R., SIGLER, P. B. & MCKNIGHT, S. L. (1989). *Science*, **246**, 911-916.
WALLACE, B. A. & RAVIKUMAR, K. (1988). *Science*, **241**, 182-187.
WOOD, S. P., TICKLE, I. J., TREHARNE, A. M., PITTS, J. E., MASCARENHAS, Y., LI, J. Y., HUSAIN, J., COOPER, S., BLUNDELL, T. L., HRUBY, V. J., BAKU, A., FISCHMAN, A. J. & WYSSBROD, H. R. (1986). *Science*, **232**, 633-636.
YANG, D. S. C., SAX, M., CHAKRABARTTY, A. & HEW, C. L. (1988). *Nature (London)*, **333**, 232-237.
ZALKIN, A., FORRESTER, J. D. & TEMPLETON, D. H. (1966). *J. Am. Chem. Soc.* **88**, 1810-1814.

Acta Cryst. (1992). B**48**, 356–369

The Perils of *Cc*: Comparing the Frequencies of Falsely Assigned Space Groups with their General Population

BY WERNER H. BAUR AND DETHARD KASSNER

Institut für Kristallographie und Mineralogie, Johann Wolfgang Goethe-Universität, Senckenberganlage 30, *W*-6000 *Frankfurt am Main, Germany*

(*Received* 11 *July* 1991; *accepted* 10 *December* 1991)

Abstract

A compilation of 221 space-group corrections from a false low symmetry (FS) to a higher true symmetry (TS) shows that higher symmetry is often overlooked in only a few space-group types. An incorrect lattice (false crystal class) is found most often for rhombohedral space-group types, and there especi-

0108-7681/92/040356-14$06.00