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An exceptionally stable peptide nanotube system with flexible pores

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The hydrophobic channels in the structure of the dipeptide L-alanyl-L-valine act as supramolecular hosts for organic solvent molecules. In a series of data collections, it is demonstrated that small molecules like acetonitrile, methanol and acetone can be removed from the channels by drying without impairing the structure of the hydrogen-bonded peptide host structure. The title compound is one of the very first organic molecules to be found to have this property. Alcohol guests larger than methanol are also absorbed, but they induce a doubling of two axes and a change in the shape and size of the pores. The observed structural modifications explain why these solvent molecules are more or less irreversibly trapped inside the channels.

1. Introduction

Compounds and materials that form nanotubes in the solid phase have been the subject of considerable research efforts in recent years (de Mendoza, 1998; Ward, 1998; Hulliger & Langley, 1999; Moriarty, 2001; Bong *et al.*, 2001; Nangia, 2001). The first peptide-based systems were described by Karle *et al.* (1975) for cyclic α-β-α-β peptides. Later on, Ghadiri and co-workers studied cyclic D,L-peptides with eight to 12 residues (Ghadiri *et al.*, 1993; Hartgerink *et al.*, 1998). Both types of molecules build tubular structures through formation of β-sheet-like intermolecular hydrogen bonds between functional groups in the peptide backbones. Other research groups have since continued to use β-amino acids (Seebach *et al.*, 1997; Clark *et al.*, 1998), but some have also used cysteine-based macrocyclic ureas (Ranganathan, Lakshmi *et al.*, 1999), bisamides (Ranganathan, Haridas *et al.*, 1999), and aromatic rings (Ranganathan *et al.*, 1998).

Recently, the formation of hydrophilic nanotubes by supramolecular aggregation of much smaller molecules was demonstrated for four dipeptides: L-Leu-L-Leu, L-Leu-L-Phe, L-Phe-L-Leu and L-Phe-L-Phe (Görbitz, 2001). The first example of a nanotube-forming dipeptide, however, was L-Val-L-Ala (Görbitz & Gundersen, 1996). The nanotubes have hydrophobic inner surfaces and are located at the axes of helices resulting from head-to-tail hydrogen bonds between the dipeptide molecules. In this paper, structural results for the retroanalogue L-Ala-L-Val are presented, with special focus on the absorption and desorption of solvent molecules in the hydrophobic channels.

2. Experimental

2.1. Crystal preparation and data collection

L-Ala-L-Val was purchased from Sigma and used as received. Crystals were prepared by dissolving about 1 mg of

the peptide in 30 ml of water with subsequent vapour diffusion of acetonitrile into the aqueous solution. A particularly large specimen was chosen for this investigation in order to maximize diffraction intensities (Görbitz, 1999). A total of nine data sets were collected for this crystal, following the procedure shown in Fig. 1. The structures resulting from refinement of data sets one, three, five and seven are presented in detail here and have been labelled (1), (2), (3) and (4), respectively. The second and the fourth data collection turned out to yield structures that were partly saturated with solvent, while the structure obtained from data set six was completely similar to structure (2) (from data set three, dry crystal), although with a slightly higher *R*-factor (0.049 *versus* 0.035). The last two data collections yielded structures that deviated very little from structure (4).

For each data set more than a hemisphere of reciprocal space was collected by a combination of five sets of exposures. Exposure times were 60 s while the crystal-to-detector distance was 5.0 cm.

acetonitrile solvate **1**

↓ 333 K, 3 days

acetonitrile solvate
(content 60% reduced)

↓ 333 K, 11 days

dry crystal **2**

↓ Soak 3 s in methanol

methanol solvate
(10% of saturation content)

↓ Soak 60 s in methanol

methanol solvate **3**

↓ 333 K, 2 weeks

dry crystal

↓ Soak 60 s in 2-propanol

2-propanol solvate hydrate **4**

↓ 333 K, 2 weeks

2-propanol solvate
(water lost, 2-propanol content as above)

↓ 353 K, 2 months

2-propanol solvate
(content slightly reduced)

Figure 1
Summary of the procedure leading to nine data collections for a single L-Ala-L-Val crystal. Bold numbers refer to refined structures described in detail in the text.

2.2. Structure determination and refinement

All structures were solved routinely by *SHELXTL* (Sheldrick, 1997). Positional parameters were refined for H atoms bonded to N and O atoms in (1) and (2). Other H atoms in (1) and (2) and all H atoms in (3) were included in theoretical positions with refinement of the distance to the bonded atom only. U_{iso} values for H atoms were $1.5U_{eq}$ (water) or $1.2U_{eq}$ (other) of the bonded atom, except that U_{iso} values were refined for the freely rotating amino and methyl groups in (1) and (2).

Refinement of (4), with four peptide molecules in the asymmetric unit, was considerably more demanding. At an early refinement stage, the structure appeared to be well defined, apart from an orientational disorder for the L-Val side chain of peptide molecule *A*. Eventually, however, it turned out that for each of the peptide molecules *A*, *B*, *C* and *D* there is a nearby (separation 0.0–1.4 Å, average 0.85 Å) alternative position called *E*, *F*, *G* and *H*, respectively. Occupancies range from 0.081 (6) for *H* to 0.125 (6) for *G*. The position of *E* relative to *A* is shown in Fig. 2. *C* and *G* have the N-terminal, the part close to the crystallographic threefold screw axis, in common. Corresponding bond lengths and bond angles within each pair of major and minor components were linked by *SHELXTL SAME* 0.005 0.01 restraints. H atoms were

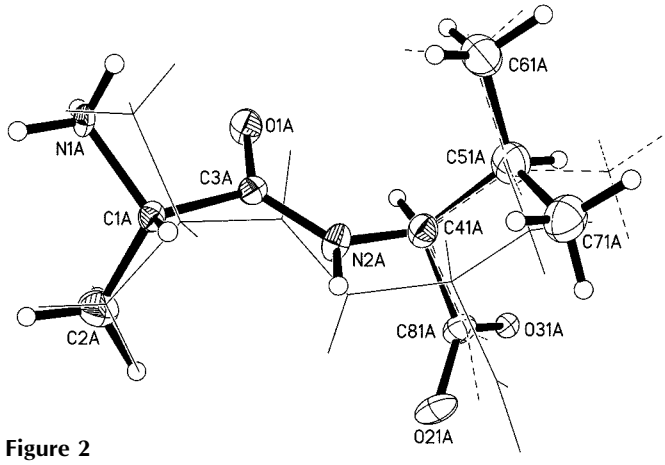

Figure 2
The structure of molecule *A* in (4). Displacement ellipsoids are shown at the 50% probability level. Ellipsoids without shaded segments for the L-Val side chain and the C-terminal carboxylate group show the major orientations (occupancy 0.53); minor orientations (occupancy 0.38) are indicated by dashed lines. A complete alternative position (occupancy 0.09), called molecule *E*, is shown as a solid line (atomic numbering as for *A*). L-Val C^{γ}-atoms were not found for *E*, and the side chain is drawn as a methyl group.

Table 1
Experimental details.

	(1)	(2)	(3)	(4)
Crystal data				
Chemical formula	$C_8H_{16}N_2O_3\cdot0.35C_2H_3N$	$C_8H_{16}N_2O_3$	$C_8H_{16}N_2O_3\cdot0.75CH_4O$	$C_8H_{16}N_2O_3\cdot0.25C_3H_8O\cdot0.22H_2O$
Chemical formula weight	202.6	188.23	212.25	206.84
Cell setting, space group	Hexagonal, $P6_1$	Hexagonal, $P6_1$	Hexagonal, $P6_1$	Hexagonal, $P6_1$
a, c (Å)	14.1635 (8), 9.8702 (5)	14.2957 (3), 9.9077 (2)	14.3102 (10), 9.8997 (7)	28.7247 (16), 9.9009 (8)
γ (°)	120	120	120	120
V (Å^3)	1714.74 (16)	1753.53 (5)	1755.7 (2)	7074.8 (8)
Z	6	6	6	24
D_x (Mg m^{-3})	1.177	1.069	1.204	1.137
Radiation type	Mo $K\alpha$	Mo $K\alpha$	Mo $K\alpha$	Mo $K\alpha$
No. of reflections for cell parameters	17994	18212	6549	56358
θ range (°)	2.65–37.03	2.63–37.0	2.63–34.96	0.82–27.10
μ (mm^{-1})	0.089	0.082	0.089	0.088
Temperature (K)	150 (2)	150 (2)	150 (2)	150 (2)
Crystal form, colour	Needle, colourless	Needle, colourless	Needle, colourless	Needle, colourless
Crystal size (mm)	1.50 × 0.40 × 0.30	1.50 × 0.40 × 0.30	1.50 × 0.40 × 0.30	1.50 × 0.40 × 0.30
Data collection				
Diffractometer	Siemens SMART CCD diffractometer	Siemens SMART CCD diffractometer	Siemens SMART CCD diffractometer	Siemens SMART CCD diffractometer
Data collection method	Sets of exposures each taken over 0.3°ω rotation scans	Sets of exposures each taken over 0.3°ω rotation scans	Sets of exposures each taken over 0.3°ω rotation scans	Sets of exposures each taken over 0.3°ω rotation scans
Absorption correction	Empirical (*SADABS*; Sheldrick, 1996)	Empirical (*SADABS*; Sheldrick, 1996)	Empirical (*SADABS*; Sheldrick, 1996)	Empirical (*SADABS*; Sheldrick, 1996)
T_{min}	0.875	0.884	0.875	0.876
T_{max}	0.974	0.976	0.974	0.974
No. of measured, independent and observed reflections	33538, 5820, 5127	35248, 5946, 5279	30692, 2677, 2357	87295, 5504, 5409
Criterion for observed reflections	$I > 2\sigma(I)$	$I > 2\sigma(I)$	$I > 2\sigma(I)$	$I > 2\sigma(I)$
R_{int}	0.0257	0.0274	0.0456	0.0364
θ_{max} (°)	37.03	37.00	34.96	27.10
Range of h, k, l	$-24 \rightarrow h \rightarrow 24$ $-22 \rightarrow k \rightarrow 23$ $-16 \rightarrow l \rightarrow 16$	$-23 \rightarrow h \rightarrow 23$ $-24 \rightarrow k \rightarrow 24$ $-16 \rightarrow l \rightarrow 16$	$-23 \rightarrow h \rightarrow 21$ $-23 \rightarrow k \rightarrow 22$ $-15 \rightarrow l \rightarrow 15$	$-36 \rightarrow h \rightarrow 36$ $-36 \rightarrow k \rightarrow 36$ $-12 \rightarrow l \rightarrow 12$
Refinement				
Refinement on	F^2	F^2	F^2	F^2
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.0352, 0.0903, 1.046	0.0358, 0.0952, 1.042	0.0562, 0.1511, 1.23	0.1006, 0.2707, 1.195
No. of reflections and parameters used in refinement	5820, 187	5946, 141	2677, 180	5504, 714
H-atom treatment	Mixed	Mixed	H-atom parameters constrained	H-atom parameters constrained
Weighting scheme	$w = 1/[\sigma^2(F_o^2) + (0.0615P)^2]$ where $P = (F_o^2 + 2F_c^2)/3$	$w = 1/[\sigma^2(F_o^2) + (0.0629P)^2 + 0.0354P]$ where $P = (F_o^2 + 2F_c^2)/3$	$w = 1/[\sigma^2(F_o^2) + (0.0790P)^2 + 0.2629P]$ where $P = (F_o^2 + 2F_c^2)/3$	$w = 1/[\sigma^2(F_o^2) + (0.0456P)^2 + 38.7799P]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max}$	0.017	0.002	0.007	0.015
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	0.429, −0.188	0.428, −0.214	0.494, −0.225	0.518, −0.556
Extinction method	None	None	None	None

Computer programs used: *SMART* (Bruker, 1998), *SAINT* (Bruker, 1998), *SHELXTL* (Sheldrick, 1997).

included in theoretical positions without refinement; U_{iso} values were fixed as for (1)–(3).

The co-crystallized acetonitrile solvent of (1) was disordered; four molecular positions (restrained geometry) were refined with occupancies from 0.054 to 0.170. The methanol solvent of (3) was modelled by eight independent C atoms (no geometry restraints) with occupancy from 0.13 to 0.27, while for (4), four positions were refined for 2-propanol solvent molecules with constrained geometry, occupancy from 0.18 to 0.37 (total 0.97). Solvent water molecules were modelled by three isotropic O atoms with occupancy from 0.24 to 0.37.

Experimental data and refinement results are summarized in Table 1.[1]

[1] Supplementary data for this paper are available from the IUCr electronic archives (Reference: OS0094). Services for accessing these data are described at the back of the journal.

Table 2
Torsion angles (°) in structures (1)–(4).

Molecule	N1—C1—C3—N2 (ψ_1)	C1—C3—N2—C4 (ω_1)	C3—N2—C4—C8 (φ_2)	N2—C4—C8—O2 (ψ_T)†	N2—C4—C5—C6 ($\chi_2^{2,2}$)	N2—C4—C5—C7 ($\chi_2^{2,2}$)
1	151.01 (6)	175.98 (6)	−130.56 (6)	−45.24 (8)	−65.79 (7)	171.72 (6)
2	152.01 (6)	176.15 (6)	−131.27 (6)	−44.61 (9)	−65.30 (8)	172.12 (7)
3	152.49 (15)	176.30 (15)	−132.66 (17)‡	−42.81 (16)‡	−65.0 (2)	172.13 (19)
4*A* (major)	156.1 (6)	174.0 (6)	−129.0 (6)	−18.0 (7)	−54.1 (18)	65.1 (12)
4*A* (minor)	156.1 (6)	174.0 (6)	−136.0 (7)	−41.8 (8)	−73 (2)	166.1 (17)
4*B*	154.6 (6)	176.8 (6)	−134.7 (6)	−16.5 (6)	−63.3 (8)	60.9 (9)
4*C*	157.7 (5)	176.0 (6)	−135.3 (5)	−39.2 (6)	−62.9 (8)	175.2 (7)
4*D*	154.1 (6)	175.0 (6)	−133.2 (6)	−44.3 (6)	−65.1 (8)	174.2 (7)
4*E*	161 (2)	−170.0 (2)	−148 (2)	−59 (3)	– §	–
4*F*	157.9 (19)	−172.3 (17)	−146.3 (18)	−69 (2)	–	–
4*G*	157.7 (5)	−163.3 (9)	−140.7 (9)	−73 (3)	–	–
4*H*	153 (2)	−174.9 (16)	−136.4 (19)	−61 (2)	–	–

† Measured to the O atom giving the smallest positive or negative value. ‡ −123.8 (4)° and −27.1 (9)° for the minor carboxylate orientation. § Side-chain atom not located.

3. Results and discussion

The crystal packing of the acetonitrile solvate (1), depicted in Fig. 3, is very reminiscent of the structure of L-Val-L-Ala, with the same hydrogen-bonding network [see Görbitz & Gundersen (1996), for a detailed discussion]. Assuming an effective van der Waals length of 4.9 Å for acetonitrile (sum of bonds 2.2 Å, sum of van der Waals radii for N and H 2.7 Å), a head-to-tail stacking in the channels would give about 2.0 molecules for a unit-cell translation of 9.9 Å (*c*-axis length). The calculated 0.35 acetonitrile molecule pr peptide (Table 1) corresponds to 2.1 solvent molecules for a unit cell. This confirms that (1) was saturated with acetonitrile.

Single crystals of L-Val-L-Ala survive a complete loss of solvent (which takes place in a matter of seconds), albeit with an obvious deterioration of crystal quality (transparent ⇒ opaque; Görbitz & Gundersen, 1996). In contrast, the drying of (1) to give (2) proceeds without visible changes to the crystal, and the two structures are virtually identical except that for (2) no electron density is found within the channels. Although coordination polymers have been found that remain single-crystal after removal of solvent, L-Val-L-Ala and L-Ala-L-Val are among the first organic structures to be found to have this property, which can be attributed to the rigid hydrogen-bonded peptide scaffolding. Brunet *et al.* (1997) found an example of a crystal that was still diffracting after 63% removal of dioxane from the host structure, while 4,4′-bipyridyl complexes have been shown to be stable after complete loss of guest molecules (Pedireddi *et al.*, 1997; Ranganathan, Pedireddi *et al.*, 1999).

Upon soaking in methanol (Fig. 1), the alcohol molecules are absorbed in the channels, giving a saturation structure (3), which is very similar to (1) and (2) (Table 2), except that some disorder is observed at the carboxylate end of (3), contributing to a higher final *R*-factor and a lower number of observed reflections for (3) than for (1) and (2) (Table 1). The major carboxylate orientation [occupancy 0.868 (6)] corresponds to the geometry in (1) and (2).

It is noteworthy that the initial drying of (1) to give (2) yielded a significantly *larger* unit cell, with cell volumes

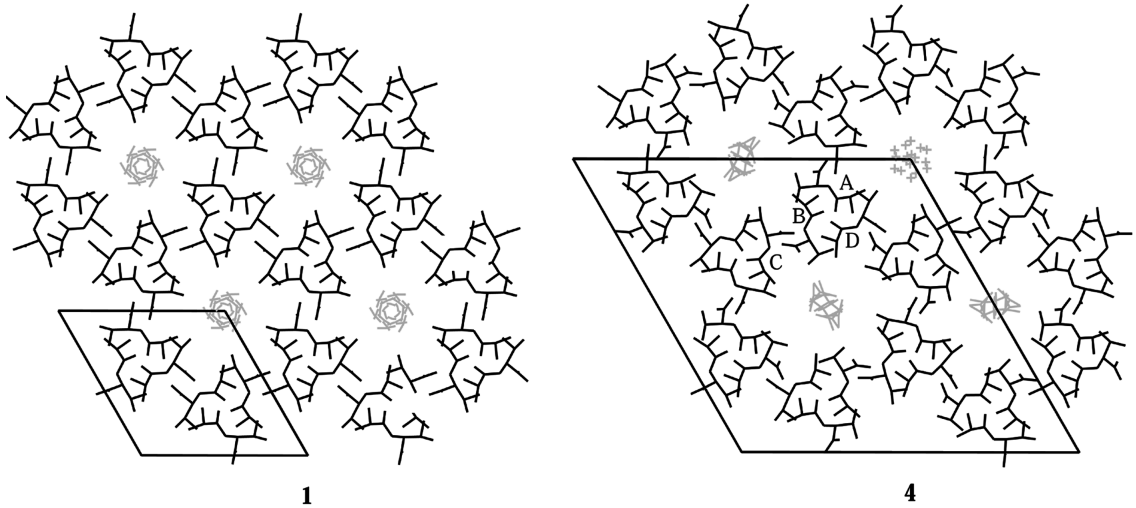

Figure 3
The unit cell and molecular packing of (1) (left) and (4) (right) viewed along the *c* axis. Solvent guest molecules are shown in grey, positions for water molecules in (4) appear as small crosses.

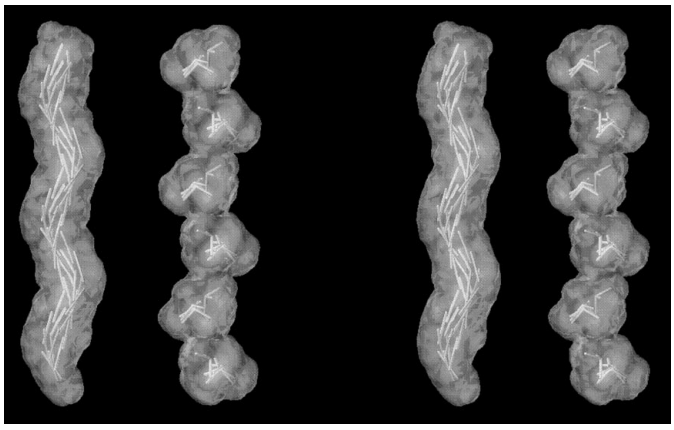

Figure 4
Stereo drawing showing a volume contour surface around disordered solvent molecules in (1) (left) and (4) (right). H atoms were included when calculating the surface, but are not displayed.

1714.74 (16) Å^3 and 1753.53 (5) Å^3, respectively. The unit cell of (3), on the other hand, is still larger, with cell volume 1755.2 (2) Å^3. The linear acetonitrile molecules are obviously easily accommodated in the channels and act as glue that serves to contract the peptide channels, while methanol requires larger channels.

After the crystal had been dried for the second time, soaking in 2-propanol made the molecular packing undergo a remarkable transformation to structure (4). The space group remains $P6_1$, but the *a* and *b* axes' lengths are roughly doubled. The new cell volume is 4.03 times the cell volume of (3), and the number of peptide molecules in the asymmetric unit accordingly increases from (1) to (4) (*A*, *B*, *C* and *D*, Fig. 3). All of them have essentially the same extended main chain conformation as already observed in (1), (2) and (3), Table 1, and Fig. 2. The L-Val side chain of molecule *B*, however, is rotated from the usual *trans*/*gauche*− conformation to a *gauche*+/*gauche*− conformation. For *A*, there is 3:2 disorder between *gauche*+/*gauche*− and *trans*/*gauche*− (Fig. 2).

Disorder in the crystal also includes the minor alternative peptide positions *E*, *F*, *G* and *H* (Fig. 2). The crystal showed visible signs of wear at the time of data collection, but the high final *R*-factor, 0.1009, is nevertheless clearly a result of this hard-to-model disorder, and not crystal cracking or other defects. This statement is based on the fact that refinement of structures from five additional data sets collected for different L-Ala-L-Val crystals grown with 2-propanol as precipitating agent showed the same unit-cell dimensions and overall structural features. These crystals were smaller, however, and the weaker diffraction patterns made it even more difficult to resolve the disorder. Accordingly, all these refinements converged at *R*-factors ≥ 0.125.

It can be seen from Fig. 3 that the channels at the hexagonal axes, generated from the L-Ala residue of molecule *A* and the L-Val residue of molecule *D*, are indistinguishable from those observed in (1)–(3). Since 2-propanol molecules evidently cannot enter such channels, it can be assumed that the observed electron density within them is due to co-crystallized water molecules from traces of water present in the alcohol used for soaking. The remaining pores have become slightly larger with distinctly elliptic cross sections (Fig. 3) as the result of minor relative translations and rotations of molecule *B*, molecule *C* and the L-Val residue of molecule *A*. Unlike the hexagonally symmetric channels, which have a fairly even diameter along the *c* axis, the ellipsoid channels are actually more like a chain of cavities in which the disordered 2-propanol molecules reside (Fig. 4). The combined occupancy for the four refined orientations is 0.97, showing that saturation has been achieved. The narrow passes between cavities explain why removal of the guest molecules is a much slower process for the 2-propanol solvate (4) than for the acetonitrile and methanol solvates (1) and (3). In essence, the crystal selectively and irreversibly traps larger solvent molecules, while small solvent molecules can move in and out.

The disorder phenomenon for the peptide molecules obviously results from the need to make room for the rather bulky 2-propanol molecules, but the details in this adaptation are unclear since L-Val side-chain atoms beyond C^β were not found for the minor peptide positions. The geometries of the peptide molecules *E*–*H* differ from those of *A*–*D* mainly at the peptide bond (ω_1) and the orientation of the C-terminal carboxylate group (ψ_T) (Table 2).

Acetonitrile was used to prepare (1), but L-Ala-L-Val crystals can also be obtained with acetone, ethanol, 1-propanol, 2-butanol and 2-methyl-2-propanol as precipitating agents [as well as 2-propanol, which gives crystals equivalent to (4)]. The structure of the acetone solvate is similar to (1) and (3). The other solvates are closely related to (4), but the refinements converged at *R*-factors around 0.13, which made a rigorous treatment of the complex disorder impossible. Some soaking tests and crystal growth experiments were also carried out for the analogue L-Val-L-Ala, for which no examples of structural changes were observed.

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