Peptide Structure Elucidation

# The Antiviral Antibiotic Feglymycin: First Direct-Methods Solution of a 1000+ Equal-Atom Structure**

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Despite all the progress in recent decades, antiviral chemotherapy is still much less effective than the treatment of bacterial infections by antibiotics. The human immunodeficiency virus (HIV) represents a particularly problematic case since its high variability is likely to reduce the effectiveness of vaccines. Although many antiviral compounds are now available for the treatment of acquired immunodeficiency syndrome, the increasing resistance of HIV requires the constant development of new drugs. The 13-amino-acid feglymycin (Figure 1), a novel peptide isolated from *Streptomyces* cultures, was found to strongly inhibit the formation of HIV syncytia in vitro, and a weak antibacterial activity against Gram-positive bacteria has also been reported.[1] Based on its unique amino acid sequence and biological activity, feglymycin represents a promising new class of antibiotics.

Feglymycin contains a high percentage of unusual amino acids such as 4-hydroxyphenylglycine and 3,5-dihydroxyphenylglycine. Structural relatives that contain hydroxyphenylglycine residues have also been found to inhibit diverse steps in the replication of HIV. In addition, a large percentage of anti-HIV agents isolated from natural sources also contains a 1,3-hydroxyphenyl moiety either detached or as part of a condensed ring system.[2]

In this communication we report the crystal structures of two crystal forms (**1** and **2**) of feglymycin. Crystals of **1** diffracted to atomic resolution (1.10 Å), and the phase problem could be solved by ab initio direct methods despite (or possibly even aided by) perfect merohedral twinning.[3] With about 1033 unique non-hydrogen atoms, this structure is about 50% larger than the largest equal-atom structure (containing no atom heavier than oxygen) previously solved by direct methods; equal-atom structures are much more resistant to solution by direct methods than structures containing a few heavier atoms. The structure of crystal form **2**, which diffracted to 1.40 Å, could be solved by molecular replacement using a feglymycin dimer (taken from **1**) as search fragment; it also exhibits perfect merohedral twinning.[3] The X-ray sequence of the peptide confirmed that deduced from NMR and MS data, and the configurations of all chiral centers could be assigned; except for the two termini, the chirality of the residues alternates between D and L.

Although crystals of **1** grew under aqueous conditions (0.1 M Tris/Tris HCl pH 8.4, 4% PEG8000) with six antibiotic molecules in the asymmetric unit, and **2** from highly alcoholic solution (0.25 M $Na_3Cit/H_3Cit$ pH 6.5, 30% isopropyl alcohol) with eight, both structures contain similar double-helical dimers that interact to form infinite helical chains along the crystallographic *c* axes. As observed for some other alternating D,L-peptides with bulky side chains, for example, the membrane channel peptide gramicidin, the feglymycin dimers are wide, antiparallel, double-stranded β helices. However the helical pitch of feglymycin varies around 9.0 residues per turn, while for the native gramicidin it is 5.6 and for gramicidin-$Cs^+$ 7.2 residues per turn.[4] Despite this structural homology, it is not likely that feglymycin acts as a membrane channel peptide: the channel is probably not long enough to span a biological membrane (Figure 2), and although the feglymycin channel is wider, it is blocked by phenylalanine side chains that would prevent all transport phenomena (see figure in the Table of Contents). Action as an ion carrier seems more feasible and would also suggest a mechanism for membrane penetration of feglymycin that may be important in the HIV-inhibitory activity.

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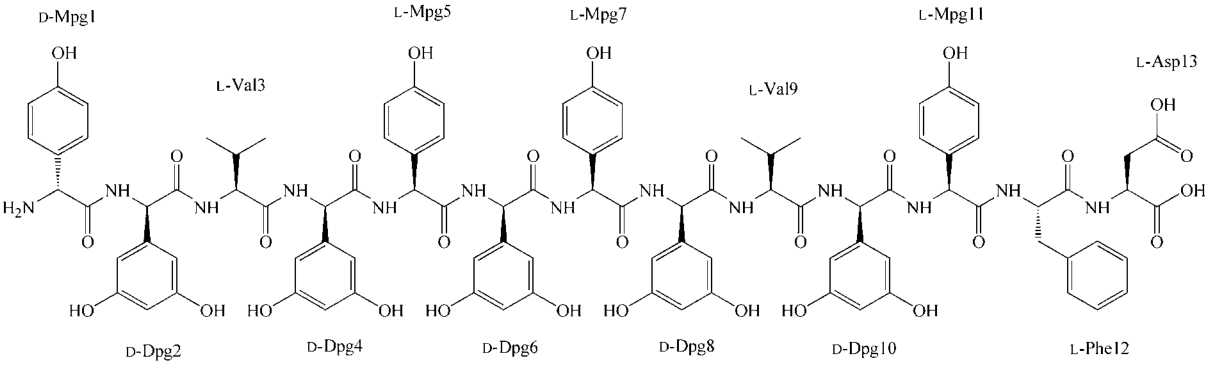

***Figure 1.*** Chemical structure of feglymycin. The absolute configuration assignment is based on the hand of the conventional amino acids, which are all present as the L enantiomer (identified by HPLC).[1] Mpg = 4-hydroxyphenylglycine, Dpg = 3,5-dihydroxyphenylglycine.

The feglymycin dimer is significantly less symmetric than those of gramicidin, which exhibit nearly exact twofold symmetry. This is to be expected since the chirality alternation for feglymycin is not as regular as for gramicidin. The most distinctive difference between the peptides constituting one dimer can be found at Phe12, where, to avoid collision of the two benzene rings, the main chain of one monomer takes a sudden 60° turn and the corresponding side chain also rotates by 120° (Figure 3). The dimer is stabilized by a multitude of secondary interactions involving hydrogen bonds either between main-chain amide hydrogens and carbonyl oxygens or between hydroxy groups of dihydroxyphenylglycine residues, thus creating two hydrogen-bonding shells. Hydrophobic interactions between the two Phe12 side chains and among the benzene rings of hydroxyphenylglycine residues may also contribute substantially to the stability and result in a rather rigid structure, as indicated by the mean-square deviations between the main-chain atoms of the six independent dimers in **1** after least-squares fitting, which vary in the range 0.1–0.2 Å. Moreover, the presence of 4-hydroxyphenylglycine residues creates a well-defined hydrogen-bonding pattern on the dimer surface, since rotation of these side chains about the Cα–Cβ bond does not change the position of their hydroxy groups.

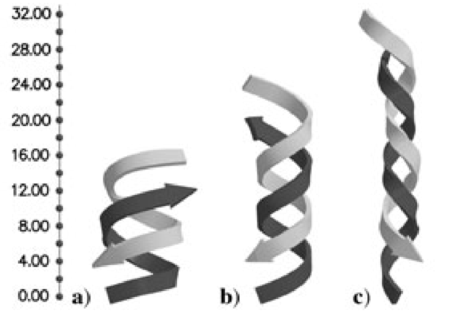

***Figure 2.*** Schematic representation of the size and structure of dimers of a) feglymycin, b) gramicidin-Cs$^+$, and c) uncomplexed gramicidin. Note that (a) and (b) are right-handed helices, while (c) is left-handed. The ruler on the left shows the scale in Å.[5]

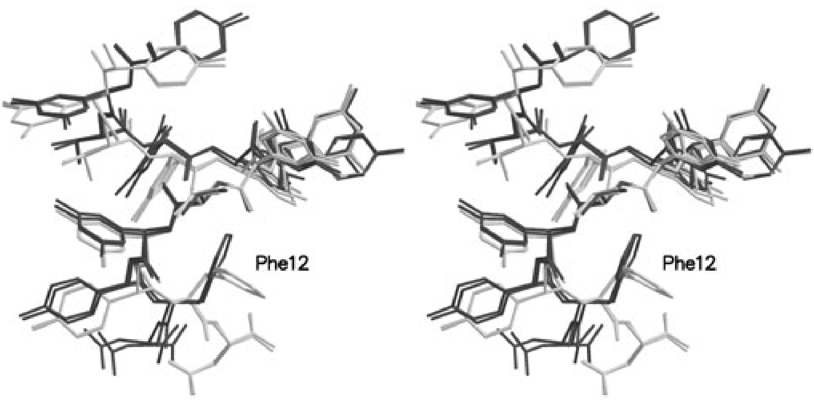

***Figure 3.*** Stereoview of the superposition of four feglymycin molecules that constitute two dimers. Rigid fragments (corresponding to residues Mpg7–Val9) were identified with ESCET and fitted using LSQKAB.[6] Although the rigid part comprises only three out of 13 residues, the main-chain conformations are fairly similar. A significant deviation starts at Phe12, where the main chain either continues as the helical structure would require (light color) or takes a sharp turn (dark color). A dimer is made up of a light-colored and a dark-colored monomer.[5]

In both crystal forms, the $6_5$ or $6_4$ crystallographic screw axes arrange the dimers into infinite helical chains. Since the dimers are not symmetric, one can distinguish two types of interdimer interfaces. One involves the interaction of the open sides of the dimers and creates a binding pocket that binds several ethyleneglycol units of a PEG molecule in **1** and two molecules of isopropyl alcohol in **2** (Figure 4); the other, between the closed sides, lacks such properties and keeps the molecules further apart. This may suggest that the biologically active species may be a tetramer formed by the association of two dimers at their more open sides that could enclose an ionic or polar species. Apart from these interfaces, very few connections can be seen between the molecules. These are limited to a small number of hydrogen bonds and hydrophobic interactions between the benzene rings and hydroxy groups of certain dihydrophenylglycine residues. There are also large holes in the crystal that are filled by disordered solvent. Therefore, though the interaction within a helical chain is relatively strong, the helices are kept together by much weaker forces, and this property is most likely to be responsible for the unusual fragility exhibited by these

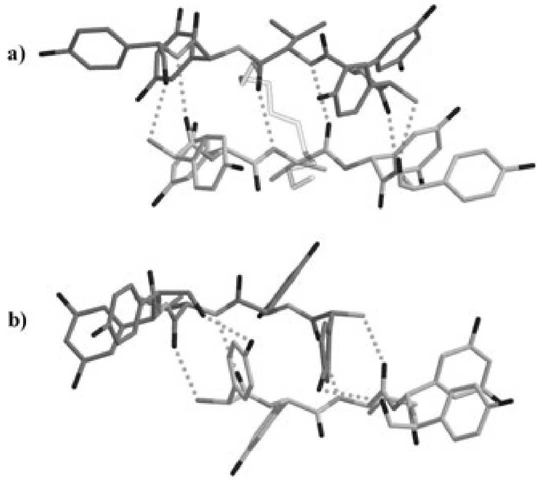

*Figure 4.* The packing of feglymycin dimers can either result in a) a binding pocket, when the dimers face each other with their more open sides or b) a more closed packing that does not allow anything to enter the internal cavity of the dimers. For clarity, only the residues involved in the interdimer interface are shown. In **1** the binding pocket is filled with a polyethylene glycol fragment (shown as a pale zigzag).[5]

crystals. In both crystal forms, the twin components are related by a rotation of 180° about an axis perpendicular to *c,* simulating the higher symmetry hexagonal Laue group; this form of twinning is not unusual for trigonal and hexagonal crystals. The twin boundary involves a reversal of the helix direction, which can arise easily because of the weak lateral interactions between the helices.

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[1] L. Vértesy, W. Aretz, M. Knauf, A. Markus, M. Vogel, J. Wink, *J. Antibiot.* **1999**, *52*, 374–382.

[2] a) T. Asano, K. Matsuoka, T. Hida, M. Kobayashi, Y. Kitamura, T. Hayakawa, S. Iinuma, A. Kakinuma, K. Kato, *J. Antibiot.* **1994**, *47*, 557–565; b) H. Tanaka, K. Matsuzaki, H. Nakashima, T. Ogino, A. Matsumoto, H. Ikeda, H. B. Woodruff, S. Ōmura, *J. Antibiot.* **1997**, *50*, 58–65; c) G. Matthée, A. D. Wright, G. M. König, *Planta Med.* **1999**, *65*, 493–506.

[3] a) Crystallographic data for **1**: $6\,C_{95}H_{97}N_{13}O_{30}+37\,C_2H_4O+5\,C_2H_5OH+79\,H_2O$, hexagonal, space group $P6_5$, $a=b=83.54$, $c=35.73$ Å, $V=215\,949.7$ Å$^3$, $Z=6$, $F(000)=44\,831$, $\lambda=1.54178$ Å, $T=100$ K, $\mu(\mathrm{Cu}_{K\alpha})=0.45\ \mathrm{mm}^{-1}$, crystal dimensions $0.3\times0.3\times0.5\ \mathrm{mm}^3$, $1.22\leq2\Theta\leq88.98°$. In total 578 199 reflections were collected, of which 57 985 were independent ($R_{int}=0.0408$, Friedel pairs merged) and employed for refinement: 9811 parameters, 13 411 restraints, $R1=\Sigma|F_o-F_c|/\Sigma F_o=0.143$ ($I>2\sigma(I)$), $wR2=[\Sigma w(F_o^2-F_c^2)^2/\Sigma wF_o^4]^{1/2}=0.344$ (all data); min./max. difference electron density $-0.32/0.47\ \mathrm{e\,Å^{-3}}$. A suitable crystal was soaked in a cryoprotectant solution consisting of the crystallization medium supplemented by 25 % glycerol and shock-frozen in a cold nitrogen stream. Intensity data were collected with a Bruker rotating anode, Osmic focusing mirrors, Bruker SMART6000 4K CCD detector with $\mathrm{Cu}_{K\alpha}$ radiation by performing six $\omega$-scans at different $2\Theta$-offsets. Raw images were integrated using SAINT,and the resulting intensities were scaled using SADABS. The structure was solved using SHELXD[7] employing real/reciprocal space recycling and peaklist optimization; b) Crystallographic data for **2**: $8\,C_{95}H_{97}N_{13}O_{30}+4\,C_3H_7OH+C_6H_8O_7+106\,H_2O$, hexagonal, space group $P6_4$, $a=b=60.30$, $c=83.75$ Å, $V=263\,724.2$ Å$^3$, $Z=6$, $F(000)=52\,846$, $\lambda=0.950$ Å, $T=100$ K, $\mu(0.95\ Å)=0.05\ \mathrm{mm}^{-1}$, crystal dimensions $0.3\times0.3\times0.5\ \mathrm{mm}^3$, $1.92\leq2\Theta\leq39.67°$. In total 484 869 reflections were collected, of which 34 149 were independent ($R_{int}=0.0634$, Friedel pairs merged) and employed for refinement: 11 147 parameters, 14 595 restraints, $R1=\Sigma|F_o-F_c|/\Sigma F_o=0.155$ ($I>2\sigma(I)$), $wR2=[\Sigma w(F_o^2-F_o^2)^2/\Sigma wF_o^4]^{1/2}=0.373$ (all data); min./max. difference electron density $-0.26/0.32\ \mathrm{e\,Å^{-3}}$. After briefly soaking in a cryoprotectant solution consisting of the crystallization medium plus 20 % 1,2-propanediol, the crystal was dipped into liquid nitrogen and mounted frozen for measurement. Data sets were collected at the BL1 beamline PSF/BESSY and processed using XDS.[8] The molecular replacement program EPMR[9] was employed for structure solution; c) Both structures have been found to be merohedrally twinned by a twofold rotation perpendicular to *c* (twin ratio: 0.51 and 0.50 for **1** and **2**, respectively). Both crystal forms have very low calculated densities (ca. $0.65\ \mathrm{g\,cm^{-3}}$) because they consist of 40–50 % disordered aqueous solvent that is not taken into account in calculating the density; such solvent contents would be typical for protein crystals with similar unit-cell sizes. The structures were refined with no intensity cutoff using SHELXL[10] and electron density maps displayed by XtalView,[11] which was also employed for hand-editing of the model. Throughout the refinement, bond length, bond angle, chiral volume and planarity restraints were imposed. All non-hydrogen atoms were refined anisotropically with suitable rigid bond, similarity, and for solvent waters, approximately isotropic restraints. Hydrogen atoms were included in later stages of the refinement. The structures have been deposited in the Protein Data Bank (www.rcsb.edu/pdb) under accession codes 1w7q and 1w7r for **1** and **2**, respectively.

[4] a) D. A. Langs, *Science* **1988**, *241*, 188–191; b) B. M. Burkhart, N. Li, D. A. Langs, W. A. Pangborn, W. L. Duax, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 12 950–12 955.

[5] Pictures were generated using Molscript (P. J. Kraulis, *J. Appl. Crystallogr.* **1991**, *24*, 946–950) and rendered with Raster3D (E. A. Merritt, D. J. Bacon, *Methods Enzymol.* **1997**, *277*, 505–524).

[6] a) T. R. Schneider, *Acta Crystallogr. Sect. D* **2002**, *58*, 195–208; b) W. Kabsch, *Acta Crystallogr. Sect. A* **1976**, *32*, 922–923.

[7] G. M. Sheldrick, H. A. Hauptman, C. M. Weeks, M. Miller, I. Usón in *International Tables for Crystallography*, *Vol. F* (Eds.: E. Arnold, M. G. Rossmann), Kluwer Academic Publishers, Dordrecht, **2001**, pp. 333–351.

[8] W. Kabsch in *International Tables for Crystallography*, *Vol. F* (Eds.: E. Arnold, M. G. Rossmann), Kluwer Academic Publishers, Dordrecht, **2001**, pp. 218–225.

[9] C. R. Kissinger, D. K. Gehlhaar, D. B. Fogel, *Acta Crystallogr. Sect. D* **1999**, *55*, 484–491.

[10] G. M. Sheldrick, T. R. Schneider, *Methods Enzymol.* **1997**, *277*, 319–343.

[11] D. E. McRee, *J. Struct. Biol.* **1999**, *125*, 156–165.