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Solid State Conformation of a Terminally Diblocked D-Methionyl-L-methionine

BY A. IMMIRZI AND P. AVENA

Istituto di Chimica delle Macromolecole del CNR, Via A. Corti 12, 20133 *Milano, Italy*

M. R. CIAJOLO

Istituto Chimico della Università di Napoli, Via Mezzocannone 4, 80121 *Napoli, Italy*

J. M. BECKER

Department of Microbiology, University of Tennessee, Tennessee 37916, *USA*

AND F. NAIDER

Department of Pure and Applied Science, The College of Staten Island, CUNY, Staten Island, New York 20402, *USA*

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BOC-D-methionyl-L-methionine-OMe exhibits in the crystalline state torsion angles φ_1, ψ_1, φ_2, ψ_2 of 71·8, −73·8, −65·0 and 38·3° respectively. The molecules are H-bonded by a double N—H···O=C linkage between translated molecules with a short repeat (5·14 Å). Side-chain conformations are compared with those of other methionine-containing peptides. The unit cell and dimensions are: monoclinic, $P2_1$, a = 14·648 (2), b = 5·139 (1), c = 14·475 (2) Å, β = 103·83 (1)°, Z = 2. X-ray analysis was carried out with direct methods and least-squares refinement down to R = 0·076 using 951 non-zero intensities and counter techniques.

Introduction

Studies from a number of laboratories show that small peptides can have important physiological properties. Such molecules serve as hormones and antibiotics (Walter & Meienhofer, 1975) and are also involved in the final stages of the digestion of proteins by mammals. For example, it is known that dipeptides and oligopeptides cross the mucosa lining of the intestine intact and are subsequently hydrolyzed to amino acids which then enter the blood (Mattews, 1972, p. 71). Oligopeptides also serve as model compounds for synthetic polypeptides and proteins. With such materials important physiochemical information was gained concerning the effect of chain length, solvent, concentration and configuration on secondary-structure formation.

Recently several studies have appeared on the conformation of methionine oligopeptides in solution (Bonora & Toniolo, 1974; Naider & Becker, 1974; Becker & Naider, 1974*a*) and methionine-containing peptides were found to be transported across the cell membrane of *Escherichia coli*, *Saccharomyces cerevisiae* and *Candida albicans* (Naider & Becker, 1975; Naider, Becker & Katzir-Katchalski, 1974; Lichliter, Naider & Becker, 1976). In all of these microorganisms the ability of the peptide transport system to recognize and transport a given peptide is dependent on the configuration of the peptide (Lichliter, Naider & Becker, 1976; Becker & Naider, 1974*b*).

We believe that further information about the conformational preferences of methionine-containing peptides may be gained from studies on well defined crystals. Recently the structure of L-methionyl-L-methionine was solved (Stenkamp & Jensen, 1975). A comparison of data from this study with X-ray studies on related compounds showed that several conformations are accessible to the side chain of methionine in the solid state. In this paper we present a structure determination on a diblocked derivative of D-methionyl-L-methionine. This compound, BOC-D-Met-L-Met-OMe, is very similar to certain derivatives used in our peptide transport studies. Details about its structure will hopefully give additional information concerning the interactions of peptides with membrane receptors involved in peptide transport. Finally the information from studies on D,L peptides may prove useful in future investigations on natural peptides which contain enantiomeric sequences such as the gramicidins, and may aid the ultimate determination of the preferred conformations of synthetic D,L polypeptides.

Preparation of crystals and X-ray measurements

tert-Butoxycarbonyl-D-methionyl-L-methionine methyl

Table 1. *Crystal data and X-ray diffraction measurements*

Crystal shape and dimensions: needles, 0·05 × 0·4 mm	Calculated crystal density: 1·24 g cm^{-3}
Instrument: Philips PW-1100 four-circle diffractometer	Absorption coefficient μ = 23·77 cm^{-1}
Radiation: Cu $K\alpha$, graphite monochromator, λ = 1·5418 Å	Scan mode: ω scan
Space group: monoclinic $P2_1$	Scan speed: 1·2 deg min^{-1}
a = 14·648 (2) Å	Scan width (constant): 1·3°
b = 5·139 (1)	Backgrounds: 2 × 15 s (0·46 × half-peak-time)
c = 14·475 (2)	Maximum sin θ/λ = 0·531 Å^{-1}
β = 103·83 (1)°	3 reflections monitored every 2 h
V = 1058·0 Å^3	1516 unique reflections ($k, l \geq 0$)
Formula: $C_{16}H_{30}N_2O_5S_2$	951 'observed' reflections, *i.e.* with $I > 2{\cdot}5\,\sigma$ with σ^2 = peak-counts + 0·46^2 background-counts
M_r = 394·55	
Z = 2, $F(000)$ = 424	

ester was prepared as previously described (Naider & Becker, 1975). Crystals of this dipeptide were prepared by dissolving the material in hot ethyl acetate and adding hexane to the cloud point. The solvent was then permitted to slowly evaporate. After several days needle-shaped crystals were observed in the test tube. These were filtered, washed with cold hexane and dried *in vacuo* over P_2O_5. Samples suitable for diffraction experiments were obtained by cutting the crystals.

X-ray intensities were measured with a single-crystal counter diffractometer (see Table 1 for relevant information). Unit-cell and crystal symmetry were determined using the standard control program with the crystal mounted with the needle axis roughly parallel to the φ spindle axis of the instrument. The accurate lattice parameters are based on precise Bragg positions for 35 strong reflections with $7° < \theta < 14°$ evaluated as centers of gravity of the diffraction profiles $I = f(\theta)$, and averaging over positive and negative θ angles. In Table 1 the crystal data and intensity measurements, together with other relevant information, are given. Absorption correction was disregarded in view of the small size of the crystal and the moderate μ value.

Structure determination and refinement

The structure was determined by means of the direct multi-solution method programmed by Germain, Main & Woolfson (1970) in the program *MULTAN*. The 400 reflections with $E > 1{\cdot}16$ and the 2000 largest Sayre relationships ($E^3 > 5{\cdot}11$) were used. A starting set with three origin-fixing, three arbitrary-phase and three fixed-phase reflections (Σ_1 formula, $P > 90\%$) were considered. For each of the 32 different solutions the tangent formula was used for the phase attribution and refinement. In spite of the rather poor and broad absolute figures of merit (five solutions with Z between 1·04 and 1·07), the first solution was determined to be correct: it produced an E map with 19 peaks acceptable as atomic positions (25 expected) besides 11 spurious ones.

The structure analysis was completed through a subsequent Fourier synthesis phasing the reflections with the 19 atoms above. Atomic positions were then refined by the block-diagonal least-squares method ($\Sigma\,\Delta F^2$ minimized with unit weights) to give $R = \Sigma\,|\Delta F|/\Sigma\,|F_o| = 0{\cdot}13$ with isotropic, and 0·09 with anisotropic thermal vibration parameters.

A difference electron density map gave random peaks of intensity less than 1·0 e Å^{-3} which did not allow H atom location. The introduction of the calculated positions of 27 H atoms [the three H atoms belonging to C(3) were not considered owing to the uncertain methyl conformation] lowered R to 0·076.

The refined atomic coordinates are listed in Table 2. Atoms were labeled (see Fig. 1) according to IUPAC rules (IUPAC–IUB Commission on Biochemical Nomenclature, 1971), except for the terminal *tert*-butoxy and methoxy groups whose atoms have arbitrary labels. The atomic scattering factors used were those of Cromer & Mann (1967) and, besides *MULTAN*, programs by Immirzi (1967, 1973) were used for all the computations.*

* Lists of structure factors and anisotropic thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 32911 (13 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

Table 2. *Atomic coordinates for the non-hydrogen atoms*

	x	y	z
N(1)	4057 (6)	2204 (19)	1899 (6)
Cα(1)	3063 (7)	1776 (24)	1858 (8)
Cβ(1)	2463 (7)	3565 (26)	1116 (8)
Cγ(1)	1376 (8)	3151 (29)	994 (9)
Sδ(1)	971 (2)	0 (0)	631 (2)
Cε(1)	1159 (10)	−157 (46)	−547 (11)
C(1)	2854 (7)	2111 (27)	2833 (7)
O(1)	2808 (6)	4324 (17)	3150 (5)
N(2)	2693 (6)	−14 (24)	3282 (6)
Cα(2)	2359 (8)	24 (32)	4156 (7)
Cβ(2)	1406 (8)	−1428 (32)	4037 (9)
Cγ(2)	666 (9)	−490 (47)	3213 (10)
Sδ(2)	−434 (3)	−1975 (16)	3083 (4)
Cε(2)	−844 (11)	−783 (75)	4009 (11)
C(2)	3097 (10)	−1111 (33)	4989 (9)
O′	2874 (8)	2352 (37)	5590 (8)
O″	3949 (6)	−514 (31)	4997 (6)
C(3)	4698 (11)	−1495 (52)	5827 (10)
C(0)	4639 (8)	226 (30)	1816 (8)
O(0)	4420 (5)	−2033 (18)	1825 (7)
O(3)	5476 (5)	1148 (17)	1767 (6)
C(4)	6251 (8)	−622 (30)	1715 (10)
C(5)	5936 (11)	−2252 (36)	819 (11)
C(6)	6528 (8)	−2315 (34)	2603 (9)
C(7)	7022 (9)	1277 (32)	1637 (16)

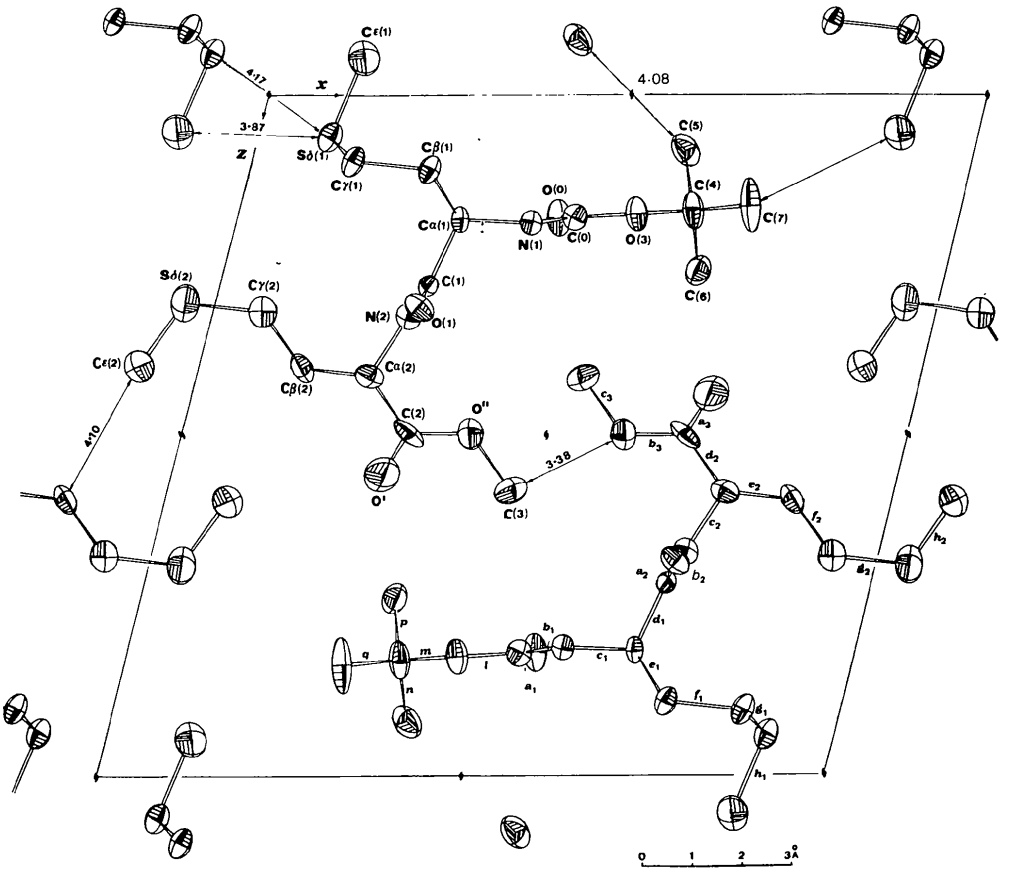

Fig. 1. View of the structure along the y axis showing thermal ellipsoids, atom and bond labeling. The shortest intermolecular distances are also indicated.

Discussion

Fig. 1 shows the *ORTEP* drawing (Johnson, 1965) of the structure viewed along the y axis. The shortest intermolecular contacts are also indicated. The structure consists of dipeptide molecules tied together in infinite chains by hydrogen bonds of the type N–H···O which link molecules repeating by a translation along the y axis. Bond lengths, valence angles and torsion angles are listed in Table 3. The bond lengths are close to expected values except for the rather short C–S distances in the side chains, particularly the Sδ(2)–Cε(2) distance (1·708 Å) which is expected to be ~1·82 Å (Kennard *et al.*, 1972). The high thermal vibration parameters of these atoms and in particular the strong U_{22} component for Sδ(2) and Cε(2) suggest that a certain conformational disorder is present, especially in the side chain of the second residue, which produces an apparent contraction in bond length (Johnson, 1970).

The backbone torsion angles φ, ψ, ω are given in Table 3 (terminal angles ω_0, φ_2, ω_2 are labeled through natural extension of IUPAC rules). While ω_0 and ω_1 are, as usual, close to 180°, the principal angles φ_1, ψ_1, φ_2, ψ_2, with values 71·8, −73·8, −65·0, 38·0°, are different from those observed in L-methionyl-L-methionine (Stenkamp & Jensen, 1975) and do not correspond to any of the structures hypothesized for alternate D,L copolypeptides by Hesslink & Scheraga (1972) and Urry, Glickson, Mayers & Haider (1972). Also the intermolecular H-bond linkage is different in the present case from that in the L-Met-L-Met case. In this study both –NH– hydrogen atoms are linked to CO groups belonging to adjacent molecules translated along the same direction (y axis) with a short repeat distance (5·14 Å). In the study of L-Met-L-Met, although the inner –NH– is similarly linked (in the z direction) with a comparable repeat distance (5·07 Å), the outer –NH_2 group is linked to a molecule repeating in a different direction (x axis) through a twofold screw

axis. Unfortunately the two cases are not strictly comparable owing to the blocking groups in the present case and particularly the BOC group which lends a more acidic character to the –NH– hydrogen. We cannot exclude, therefore, the possibility that the differing backbone conformation is merely a consequence of the different intermolecular H-bonding induced by the blocking groups.

The conformations of the two side chains $-CH_2-CH_2-S-CH_3$ are different (see χ's in Table 3). That of the first residue is similar to the one observed in crystals of L-methionine *b* form (Torii & Iitaka, 1973; L instead of D configuration accounts for change in signs of torsion angles); while that of the second residue is similar to the one observed in crystals of racemic D,L-methionine (Geddes, Hamodrakas & Sheldrick, 1974). In Stenkamp & Jensen (1975) the conformations of the methionine side chains observed in several X-ray studies have been summarized. It is evident that numerous conformations are easily accessible to this group and this is further confirmed by the occurrence of polymorphs in various cases. In our study the abnormal thermal-vibration parameters for the side-group atoms are also indicative of a large conformational freedom.

Examination of the intermolecular contacts (see Fig. 1) shows that besides the H-bonds which tie together the molecule along the *y* direction, the only critical contact involves the blocking OCH_3 group [C(3)···O'' 3·38 Å]. No critical contacts are observed for side-chain atoms. Of the two side chains, that having higher thermal disorder (No. 2) has also fewer intermolecular short contacts. This further confirms the conformational non-rigidity of the methionine side chains. We believe that the large number of conformations available to the side chains of methionine peptides in the solid state must be considered in using this information to make any proposal on the conformation of oligomethionines in solution.

Another interesting aspect of the present structure is that D-Met-L-Met may serve as a model for poly(D-methionyl-L-methionine). This polymer has been studied by Dellacherie, Néel & Cesari (1975) who have proposed a β-antiparallel folded structure based on oriented fiber spectra. In fact the most important feature of the spectrum is a strong meridional reflection with interplanar spacing d = 4·70 Å. This distance equals the distance N(1)···O'' in the present structure. Since in the polymer O'' is replaced by the N belonging to the next residue, it seems reasonable to suggest that the *d* of the strong meridional reflection corresponds to the 'length' of a D,L pair of residues which maintains the same relative conformation in the polymer as that which we observed in the dipeptide. The whole chain might be generated through a helix close to 2_1 (see Fig. 2) and the polymer would have a repeat of 2 × 4·70 Å. This polymer conformation would be close to the Ramachandran D,L ribbon (Ramachandran & Chan-

Table 3. *Bond lengths* (Å) *and principal bond and torsion angles* (°) (*see Fig.* 1 *for bond labeling*)

Bond lengths

a_1	1·206 (18)	g_1	1·761 (17)	e_2	1·555 (21)	c_3	1·508 (20)
b_1	1·351 (18)	h_1	1·792 (22)	f_2	1·487 (22)	l	1·332 (14)
c_1	1·459 (14)	a_2	1·234 (12)	g_2	1·751 (18)	m	1·471 (16)
d_1	1·525 (20)	b_2	1·320 (12)	h_2	1·708 (37)	n	1·521 (34)
e_1	1·522 (15)	c_2	1·462 (18)	a_3	1·186 (14)	p	1·524 (18)
f_1	1·573 (17)	d_2	1·530 (20)	b_3	1·282 (18)	q	1·518 (19)

Principal bond angles

a_1b_1	123·2 (5)	a_2b_2	123·2 (6)	$d_1b_2 = \tau(C_1)$	117·5 (5)
$b_1c_1 = \tau(N_1)$	121·8 (6)	$b_2c_2 = \tau(N_2)$	123·4 (5)	d_1a_2	119·2 (5)
$c_1d_1 = \tau(C_1\alpha)$	111·3 (5)	$c_2d_2 = \tau(C_2\alpha)$	110·9 (5)	$d_2a_3 = \tau(C_2)$	121·1 (4)
d_1e_1	112·0 (5)	d_2e_2	110·5 (5)	d_2b_3	114·7 (5)
c_1e_1	109·9 (4)	e_2e_2	112·0 (5)	a_3b_3	124·1 (5)
$e_1f_1 = \tau(C_1\beta)$	113·3 (4)	$e_2f_2 = \tau(C_2\beta)$	113·4 (6)	b_3c_3	116·6 (6)
$f_1g_1 = \tau(C_1\gamma)$	114·7 (6)	$f_2g_2 = \tau(C_2\gamma)$	114·9 (6)		
$g_1h_1 = \tau(S_1\delta)$	102·0 (5)	$g_2h_2 = \tau(S_2\delta)$	104·3 (6)		

Principal torsion angles (σ_{avg} = 1°)

$lb_1c_1 = \omega_0$	172·8	$c_2d_2b_3 = \psi_2$	38·3	$f_1g_1h_1 = \chi_1^3$	−66·4
$b_1c_1d_2 = \varphi_1$	71·8	$c_2d_2a_3$	−144·5	$c_2e_2f_2 = \chi_2^1$	−53·4
$c_1d_1b_2 = \psi_1$	−73·8	$d_2b_3c_3 = \omega_2$	177·4	$e_2f_2g_2 = \chi_2^2$	−177·3
$d_1b_2c_2 = \omega_1$	−171·4	$c_1e_1f_1 = \chi_1^1$	177·7	$f_2g_2h_2 = \chi_2^3$	70·7
$b_2c_2d_2 = \varphi_2$	−65·0	$e_1f_1g_1 = \chi_1^2$	−60·3		

Hydrogen-bond distances ($t+$ denotes translation along $+y$, $t-$ along $-y$)

N(1)···O(0)$^{t+}$	3·015	N(2)···O(1)$^{t-}$	2·924

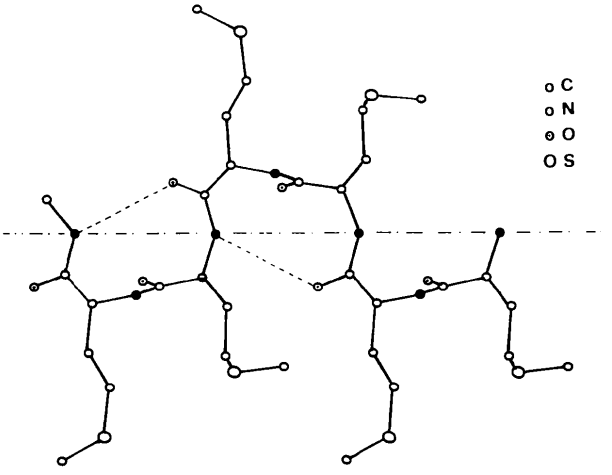

Fig. 2. Model for poly(D-methionyl-L-methionine). The relative conformation of D and L residues is that observed in BOC-D-Met-L-Met-OCH_3 and the whole chain is generated through a twofold helix. Dashed lines indicate probable intramolecular H-bonds involving 50% of the NH groups. The other NH would be involved in intermolecular H-bonds with adjacent translated chains.

drasekharan, 1970) with formation of intramolecular H-bonding (10 atom ring) for 50% of the NH groups and of intermolecular H-bonding for the other 50%.

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