Crystal Structure of Fragments of Elastin Sequence (Boc-Ala-Pro-OBzl)

Beeranahally H. DORESWAMY,* Madegowda MAHENDRA,* K. ABIRAJ** D. CHANNEGOWDA,**
Sridhar M. ANANDALWAR,* and Javaregowda S. PRASAD*†

*Department of Studies in Physics, University of Mysore, Manasagangotri, Mysore 570 006, India

**Department of Studies in Chemistry, University of Mysore, Manasagangotri, Mysore 570 006, India

Fragments of elastin sequences of mammalian elastic protein elastin, like Ala-Pro, was synthesized and characterized by X-ray crystallography. The sequence (C₂₀H₂₈N₂O₅) crystallizes in the orthorhombic space group *P*2₁2₁2₁ with the parameters *a* = 9.4240(9)Å, *b* = 11.1850(5)Å, *c* = 20.0800(19)Å, *V* = 2116.6(3)Å³, *Z* = 4. The final residual factor is 0.0612.

(Received September 8, 2003; Accepted February 2, 2004; Published on Web March 18, 2004)

Elastic protein-based polymers have their origins in repeating sequences of the mammalian elastic protein.¹ The parent polymers, (Val-Pro-Gly-Val-Gly)_{*n*} or poly(Val-Pro-Gly-Val-Gly), (Ala-Pro-Gly-Val-Gly-Val)_{*n*} or poly(Ala-Pro-Gly-Val-Gly-Val), (Val-Pro-Gly-Gly)_{*n*} or poly(Val-Pro-Gly-Gly) and (Val-Pro-Gly-Phe-Gly-Val-Gly-Ala-Gly) or poly(Val-Pro-Gly-Phe-Gly-Val-Gly-Ala-Gly), derive from sequences that occur in all sequenced mammalian elastin protein.² In the most striking

example, the sequence (Val-Pro-Gly-Val-Gly)_{*n*} occurs in bovine elastin with *n* = 11 without a single substitution.^{1a,3} Another repeat first found in porcine elastin is (Val-Pro-Gly-Gly)_{*n*}; this repeat has not been found to occur with *n* > 2 without substitution.³ The monomers, oligomers and high polymers of these repeats have been synthesized and conformationally characterized.⁴ These polymers have a number of medical and non-medical applications.⁵ In view of the above, it is of interest to investigate the crystal structure of Boc-Ala-Pro-OBzl (Fig. 1).

To Boc-Ala-OH (20 mmol), dissolved in acetonitrile (35 mL) and cooled to 0°C, was added *N*-methylmorpholine (NMM) (2.2

Fig. 1 Schematic diagram of the molecule.

Table 1 Crystallographic data

Formula: C ₂₀ H ₂₈ N ₂ O ₅
Formula weight = 376.44
Crystal system: orthorhombic
Space group: <i>P</i> 2 ₁ 2 ₁ 2 ₁ <i>Z</i> = 4
<i>a</i> = 9.4240(9)Å
<i>b</i> = 11.1850(5)Å
<i>c</i> = 20.0800(19)Å
<i>V</i> = 2116.6(3)Å ³
<i>D</i> _x = 1.181 Mg/m ³
2θ _{max} = 45.96° with Mo Kα
No. of reflections used = 2458 [<i>I</i> > 2σ(<i>I</i>)]
<i>R</i> ₁ = 0.0612
(Δ/σ) _{max} = 0.001
(Δρ) _{max} = 0.523 eÅ ⁻³
(Δρ) _{min} = -0.602 eÅ ⁻³
Measurements: DipLabo Kappa(IP)
Program system: Denzo and Scalepak
Structure determination: SHELXS-97
Refinement: SHELXL-97

Table 2 Atomic coordinates and equivalent thermal parameters

Atom	<i>x</i>	<i>y</i>	<i>z</i>	<i>U</i> _{eq}
O20	0.5919(3)	0.8195(2)	0.24178(11)	0.0731(7)
O19	0.4733(3)	0.8261(2)	0.33798(11)	0.0785(7)
N13	0.3711(3)	0.7802(2)	0.16792(13)	0.0625(7)
N8	0.3880(3)	0.5086(2)	0.07296(13)	0.0712(8)
C18	0.4754(4)	0.8208(3)	0.27813(15)	0.0621(8)
C11	0.4031(4)	0.6641(3)	0.15687(15)	0.0641(8)
C17	0.3435(4)	0.8198(3)	0.23544(15)	0.0642(8)
O5	0.2156(3)	0.3796(2)	0.05734(13)	0.0855(8)
C6	0.2556(5)	0.4961(3)	0.05119(16)	0.0728(9)
O12	0.4009(4)	0.5921(2)	0.20234(11)	0.0847(9)
C22	0.8349(4)	0.8202(3)	0.22099(18)	0.0732(9)
C21	0.7274(4)	0.8298(4)	0.27465(18)	0.0783(10)
C10	0.4445(4)	0.6274(3)	0.08644(16)	0.0703(10)
C14	0.3821(5)	0.8792(3)	0.12016(19)	0.0787(10)
O7	0.1824(4)	0.5742(3)	0.02955(17)	0.1016(10)
C27	0.9228(5)	0.7205(4)	0.2197(3)	0.0957(13)
C15	0.3665(6)	0.9891(3)	0.1635(2)	0.0970(14)
C16	0.2875(5)	0.9467(4)	0.2241(2)	0.0891(13)
C9	0.6038(5)	0.6272(4)	0.0809(2)	0.0921(12)
C3	0.0808(8)	0.2071(5)	0.0513(4)	0.139(2)
C4	0.0752(5)	0.3391(4)	0.0363(2)	0.1039(16)
C23	0.8423(6)	0.8994(5)	0.1710(2)	0.1056(14)
C24	0.9342(7)	0.8784(7)	0.1155(3)	0.1211(18)
C26	1.0131(6)	0.7010(5)	0.1665(3)	0.1175(18)
C25	1.0124(6)	0.7798(6)	0.1150(3)	0.1120(16)
C2	0.0595(10)	0.3565(7)	-0.0370(3)	0.168(3)
C1	-0.0353(7)	0.3984(8)	0.0796(5)	0.184(4)

† To whom correspondence should be addressed.
E-mail: jsp@uomphysics.net.

$$[U_{eq} = (1/3)\sum_i \sum_j U_{ij}(a_i^* a_j^*)(a_i a_j)]$$

Table 3 Bond lengths (Å)

Atom	Length	Atom	Length
O20-C18	1.318(4)	C6-O7	1.195(5)
O20-C21	1.442(5)	C22-C23	1.341(6)
O19-C18	1.203(4)	C22-C27	1.389(6)
N13-C11	1.351(4)	C22-C21	1.483(5)
N13-C17	1.450(4)	C10-C9	1.505(6)
N13-C14	1.469(4)	C14-C15	1.513(5)
N8-C6	1.330(5)	C27-C26	1.384(7)
N8-C10	1.457(5)	C15-C16	1.503(7)
C18-C17	1.510(5)		
C11-O12	1.218(4)		
C11-C10	1.523(4)		
C17-C16	1.531(5)		
O5-C6	1.362(5)		
O5-C4	1.462(5)

Fig. 2 ORTEP diagram of the molecule at the 50% probability.

mL, 20 mmol). The solution was cooled to $-15^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and isobutylchloroformate (2.6 mL, 20 mmol) was added under stirring while maintaining the temperature at -15°C . After stirring the reaction mixture for 10 min at this temperature, a pre-cooled solution of HOBt (3.1 g, 20 mmol) in DMF (30 mL) was added. The reaction mixture was stirred for an additional 10 min and a pre-cooled solution of HCl-H-Pro-OBzl (4.85 g, 20 mmol) and NMM (2.2 mL, 20 mmol) in DMF (40 mL) was added slowly. After 20 min, the pH of the solution was adjusted to eight by the addition of NMM, and the reaction mixture was stirred over night at room temperature. Acetonitrile was removed under reduced pressure, and the residual solution was poured into about 400 mL of an ice-cooled 90% saturated KHCO_3 solution, and stirred for 30 min. The precipitate was filtered, washed with water, cold 1 M HCl, water and then dried. The crude peptide was recrystallized from ether and petroleum ether to obtain 6.87 g of Boc-AP-OBzl (yield, 91.2%). R_1 , 0.50; R_2 , 0.63. $[\alpha]_D^{25}$ (C,1; MeOH)- 13.4, m.p. 67°C .

Single crystal of $0.3 \times 0.25 \times 0.2$ mm was chosen for X-ray diffraction studies. The measurements were made on a DIPLabo Imaging Plate system with graphite-monochromated radiation ($\text{Mo K}\alpha$). The structure was solved and refined using the maXus⁶ program. All the non-hydrogen atoms were revealed in the first map. A full-matrix least-squares refinement based on 2458 observed reflections ($I > 2\sigma(I)$) with isotropic temperature factors for all the atoms converged to a residual of $R = 0.1397$. A refinement of non-hydrogen atoms with anisotropic thermal parameters was started at this stage. After eight cycles of refinement, the residuals saturated at $R = 0.0612$. The hydrogen atoms were placed at calculated positions, and included using the riding atom model. Table 1 gives the details of the crystal and experimental data. Absolute configuration has been assigned by reference to an unchanging chiral centre in the synthetic procedure.

The final positional coordinates along with equivalent isotropic temperature factors for all of the non-hydrogen atoms are given in Table 2. Table 3 gives the bond distances of the non-hydrogen atoms, respectively. The bond lengths and angles are in good agreement with their standard values. Figure 2 represents an ORTEP diagram of the molecule with thermal ellipsoids at 50% probability. The packing diagram of the molecule shows stacking when viewed along the a axis. The dihedral angle between the plane of atoms, C22 to C27, and that of atoms N13 to C17 is $27.1(3)^{\circ}$, with a maximum deviation of $0.016(6)\text{\AA}$ for C26, $0.192(5)\text{\AA}$ for C15 and $0.161(4)\text{\AA}$ for C17 respectively. The structure has both intra and intermolecular

hydrogen bonds, which are as follows: C2-H20A...O7 ($3.009(8)\text{\AA}$, 119°) and N8-H16...O19 ($3.012(3)\text{\AA}$, 141°), C21-H7B...O12 ($3.207(5)\text{\AA}$, 135°), C25-H25...O7 ($3.286(7)\text{\AA}$, 134°) with symmetry codes $(1-x, -1/2+y, 1/2-z)$, $(1-x, 1/2+y, 1/2-z)$, $(1+x, y, z)$ respectively.

The bond lengths and angles of the peptide link are in conformity with those of similar molecules. The peptide bond between C11-N13 is $1.351(4)\text{\AA}$ and between C11-O12 is $1.218(4)\text{\AA}$. The values are as expected in peptide linkages. The conformation of the dipeptide is identified as follows: the rotations about C10-C11, N8-C10 and C11-N13 are, respectively, $\psi = -143.7(3)^{\circ}$, $\phi = 85.2(4)^{\circ}$ and $\omega = -174.6(3)^{\circ}$.

Acknowledgements

The authors would like to express their thanks to DST, Government of India for financial assistance under the project SP/I2/FOO/93.

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