Solid State Conformational Investigation of Boc(L-Cys(Me))$_7$OMe by X-rays

Paola Spadon,* Daniel R. Rueda, and Antonio Del Pra

Biopolymer Research Centre, C.N.R., Institute of Organic Chemistry, University of Padua, 35100 Padova, Italy. *Received April 16, 1979*

ABSTRACT: The solid state conformation of Boc(L-Cys(Me))$_7$OMe has been investigated by X-rays. X-ray fiber diagrams indicate that the samples assume a structure of the cross-β type as is already found in some synthetic polydisperse polypeptides of rather low molecular weight, in linear peptides, and in natural fibrous proteins. The unit cell is pseudomonoclinic with dimensions: a = 9.60, b = 30.0, c = 9.47 Å, α = 105.0°. As regards chain orientation, an appreciable proportion of the heptamer structure is in an antiparallel orientation.

In recent years, substantial work has been carried out in our laboratory on the synthesis and conformational analysis in solution and in solid state of complete series of monodisperse linear homo-oligopeptides derived from hydrocarbon and sulfur-containing amino acid residues having the general formula Boc(L-X)$_x$OMe,[13] where X = alanine, leucine, valine, norvaline, phenylalanine, methionine, methylcysteine, and β-cyclohexylalanine, and x = 2–7.

By CD, IR, and UV techniques, evidence was found that the ordered secondary structure generally assumed by these low molecular weight peptide molecules is of the β-pleated sheet type,[1-6] similar to that found for [Cys(Cbz)]$_n$.[7]

The present paper deals with a solid state conformational investigation on the heptamer of the *S*-methylcysteine series.

Experimental Section

Materials. The synthesis of the monodisperse homo-oligopeptides derived from *S*-methylcysteine has been carried out by Toniolo et al., according to procedures already published.[8] The compounds are chemically and optically pure.

Method. Oriented films of Boc(L-Cys(Me))$_7$OMe were obtained by stroking out a drying film until it was solid; fibers were also drawn by means of pincers from a viscous solution of the samples. Solvents used were benzene and 2,2,2-trifluoroethanol. Films and fibers were dried in vacuo at room temperature for several hours to remove free solvent molecules. The observed density, determined by the flotation method, was D_0 = 1,34 g cm^{-3}.

Films and fibers were examined in a flat camera with a sample to photographic film distance variable from 40 to 100 mm, with Ni filtered Cu K$\bar{\alpha}$ radiation, from a fine and normal focus X-ray tube and a 0.6-mm pinhole.

Tilted specimen and equi-inclination techniques were also used. Molecular models were built up from CPK space-filling components (1 Å = 1.25 cm).

Results and Discussion

Diffraction pictures have shown that the sample is crystalline and sufficiently oriented for analysis.

An X-ray photograph of Boc(L-Cys(Me))$_7$OMe is shown in Figure 1. From experimental data, clear evidence was obtained that its structure is of the cross-β type.[9] Specimens prepared as described in the experimental section, mounted with the film plane horizontal and the stroking direction normal to the X-ray beam, give diffraction patterns whose main features are a strong arc on the meridian at 4.80 Å and a reflection on the equator at 9.15 Å. The former reflection is related to the distance between hydrogen-bonded peptide chains in a sheet (a axis of the unit cell); the latter one is related to the distance between consecutive sheets (c axis). The b axis, which depends on the nearly-extended chain repeat, is perpendicular to the direction of stroking. The spacings, d_0, and eye-estimated intensities, I_0, of the observed reflections are reported in Table I. With these data, we tried to define the unit cell dimensions. The equatorial reflection at about 29 Å can be directly related to the molecular chain length.

Table I[a]

hkl	d_c, Å	d_o, Å	I_o
	Equator		
010	28.98	28.9	ms
020	14.49	14.55	w
001	9.15	9.15	vs
031	5.92	5.91	vw
032	3.77	3.78	vw
042, 07$\bar{2}$	3.48, 3.58	3.53	m
003	3.05	3.05	ms
072, 033, 08$\bar{3}$	2.74, 2.71, 2.70	2.74	w
043, 0,10,1, 09$\bar{3}$	2.58, 2.58, 2.57	2.58	w
	First Layer Line		
141	4.49	4.50	m
12$\bar{2}$	3.75	3.78	vw
16$\bar{2}$, 132	3.56, 3.51	3.53	m
	Second Layer Line		
200	4.80	4.80	vvs
231	3.73	3.80	w
	Third Layer Line		
300	3.20	3.20	w
	Fourth Layer Line		
400	2.40	2.40	ms
45$\bar{1}$, 40$\bar{2}$, 412	2.11, 2.12, 2.10	2.11	vw
43$\bar{2}$, 44$\bar{2}$	2.13, 2.11		
461, 47$\bar{0}$, 422	2.04, 2.08, 2.07	2.05	vw
432, 46$\bar{2}$	2.03, 2.04		
	Sixth Layer Line		
600	1.60	1.60	mw

[a] *t*-Boc-(L-Cys(Me))$_7$-OMe. Monoclinic unit cell, a = 9.60, b = 30.0, c = 9.47 Å and α = 105.0°. Abbreviations used: s = strong, m = medium, w = weak, v = very.

An important feature of the equi-inclination X-ray photograph is a sharp meridional reflection at 3.20 Å, which must be indexed as 300.

We found that all observed reflections can be satisfactorily indexed according to a pseudomonoclinic unit cell with a = 9.60, b = 30.0, c = 9.47 Å and α = 105°.

The length of the a axis is approximately twice the normal distance between adjacent chains in a β structure and indicates that they are antiparallel or that an appreciable proportion of the structure has an antiparallel orientation. The sharpness of the $h0l$ reflections compared to that of those in which k is not zero suggests some disorder along the b axis; this can be due to the presence of the bulky N-blocking group which can hinder a good packing between neighboring sheets. Consequently, a decrease of crystalline coherence along the b axis must be expected, which is also in agreement with the presence of few broad $0k0$ reflections.

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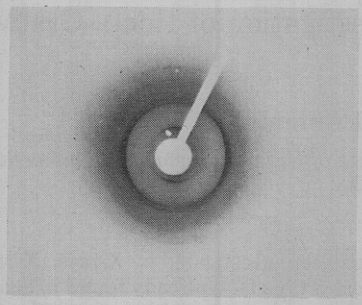

Figure 1. Normal beam X-ray diffraction pattern of *t*-Boc(L-Cys(Me))$_7$OMe oriented film; flat camera; d = 40 mm, Cu K$\bar{\alpha}$ radiation; *t*-Boc(L-Cys(Me))$_7$OMe film and stroking direction horizontal.

The c axis length is close to those found for the pentamer and hexamer of the same oligopeptide series.[10,11]

With the aid of the molecular models, we build up the antiparallel chain structure for *t*-Boc(L-Cys(Me))$_7$OMe; the peptide chain length (28 Å) is slightly shorter than the observed spacing (28.9 Å).

As far as the mode of packing of the pleated sheets is concerned, the great intensity of 200 and 001 reflections shows that all chains lie on or very near the lattice points formed by the simple cell of sides $a/2$ and c. This packing is the same already suggested by Warwicker for the silk fibroin structure.[12]

The calculated crystal density is $D_c = n0.599$ g cm^{-3}, where n is the number of peptide chains crossing the monoclinic cell. Comparison with the measured value (D_0 = 1.34 g cm^{-3}) indicates that such a cell is crossed by two β chains only. The difference between the observed and the calculated densities is undoubtedly outside the limit of the experimental error and can only be explained by the presence of solvent molecules trapped in the borderline surface of the lamellar crystallites since stringent drying conditions have not been used.

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(13) *t*-Boc = *tert*-butoxy; OMe = methoxy; Cys(Me) = *S*-methylcysteine; Cys(Cbz) = *S*-carbobenzoxy-L-cysteine.

Static and Dynamical Properties of Polystyrene in *trans*-Decalin. 3. Polymer Dimensions in Dilute Solution in the Transition Region*

T. Nose‡ and B. Chu*

Department of Chemistry, State University of New York at Stony Brook, Long Island, New York 11794. *Received June 25, 1979*

ABSTRACT: Static and dynamical properties, in terms of polymer dimensions, such as the radius of gyration (r_g) and the hydrodynamic radius (r_h), and intermolecular interactions, such as the second virial coefficients (A_2, k_D, k_f), of a high molecular weight polystyrene ($M_w = 1.2 \times 10^7$) in *trans*-decalin in the dilute solution region have been investigated. We present quantitative experimental data on the temperature (T) and some molecular weight (M_w) dependence of $\bar{r}_h$ and $\langle r_g^2 \rangle_z^{1/2}$ over the transition region before the asymptotic simple power laws become valid. In the excluded volume regime for very large polymer coils dissolved in a good solvent, the blob theory of chain statistics is in very good agreement with experiments. Our data should provide experimental boundary conditions for the modification of the blob theory, even though such changes are likely to destroy the discontinuous jump from the Gaussian to the excluded volume behavior in the blob model. We also present experimental data on the contraction of polymer coils and interaction changes below the Θ temperature. Collapse of polymer coils is of interest and requires further study.

The scaling theories[1-10] have been quite successful in describing the static and dynamical properties of polymer solutions, especially in the limit $N \to \infty$, where N is the number of monomers per chain. Earlier experiments on both static properties of entangled polymer coils in the semidilute solution by small-angle neutron scattering[3] and dynamical properties of polymer (polystyrene) solutions in a good solvent (benzene) by laser light scattering[11-13] have been in reasonable agreement with predictions of the scaling laws. Furthermore, small deviations between observation and theory are often within the error limits of the experiments. However, it should be noted that with very high molecular weight polymer samples dissolved in a good solvent most reported experiments were designed

* Author to whom requests for reprints should be addressed.
‡ On leave of absence from the Department of Polymer Chemistry, Tokyo Institute of Technology.
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