Helix Assembly in Synthetic Polypeptides. 1. Poly(γ-methyl L-glutamate) Film Retaining Cholesteric Twisted Structure

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ABSTRACT: A positionally disordered supermolecular structure in a synthetic polypeptide film was analyzed by X-ray diffraction. The helically twisted arrangement of the α-helices of poly(γ-methyl L-glutamate) (PMLG), formed in the lyotropic cholesteric liquid crystal, was retained in the film (I) prepared from the solution in 1,2-dichloroethane. In contrast to the highly crystalline film (II) prepared from the solution in chloroform, the X-ray diffraction pattern of I was broad but indicative of a particular intermolecular association. A stoichiometric complex with methanol (C), exhibiting sharp reflections, was formed for I but not for II. In the hexagonal unit cell of C, 12 or 13 helices were contained with a statistical disorder. The helices were delicately assembled so that the neighboring helices pointing in the same direction were separated by 13.2 Å while the antiparallel helices were separated by 11.1 Å. The methanol molecules were incorporated between the parallel helices. The disordered structure of I resulted from the reduction of the 13.2-Å distances to ca. 12 Å.

Introduction

Synthetic polypeptides from cholesteric liquid crystals in solutions.[1,2] The principal axis is normal to the successively twisted layers of the rodlike α-helical molecules. The twisting angle between layers increases or the pitch of the torsion decreases as the polymer concentration increases.[1] When the solvent is evaporated, the twisted structure is possibly retained in the solid film with a pitch P comparable to the wavelength of visible light. Such films will show irridescent colors associated with the form circular dichroism.[3-5] These observations have been reported, for instance, for the film (I) of poly(γ-methyl L-glutamate) (PMLG)[6-8] prepared from the solution in 1,2-dichloroethane (DCE) and the film of poly(γ-benzyl L-glutamate) (PBLG)[7-9] prepared from the solution in *N,N*-dimethylformamide (DMF). The α-helices and the cholesteric structures were right-handed, and the cholesteric axis was directed to the normal of the film surface. These films exhibited yellow or blue cholesteric color, which was due to the selective reflection of right circularly polarized light with wavelengths $\sim nP$ (n = refractive index).[7,8]

The lyotropic liquid crystal forms a visible monodomain, whereas the domains in the solid state are at most of the order of a few hundred angstroms. Therefore, the twisted structure in the solid state comprises mosaic blocks; the torsion may exist only between blocks or, alternatively, between molecular layers in each block. In the latter case, the twisting angle between successive layers, $2\pi D/P$, will be 1–2° (D = distance between layers),[7] and this may be enough to destroy the crystalline order.

The diffraction patterns from uniformly twisted models comprised of cylindrical straight rods have been numerically calculated.[10] The patterns display Bragg reflections from the layers normal to the cholesteric axis and all other broad reflections or streaks. The application of this calculation is limited. The persistence length of the α-helix of PMLG or PBLG is of the order of 1000 Å.[11] Since the persistence length corresponds to half the Kuhn segment length,[12] the molecules of PMLG with molecular weight about 10^5 are not strictly straight over the contour length about 1000 Å. If the rods were straight and rigid, the cholesteric torsion might cause the local molecular packing to change smoothly along the chain axis, for instance, from hexagonal to orthorhombic. Even if the hexagonal part is optimized, the orthorhombic part will have unreasonable interchain distances. In order to avoid this, the chains and the lattice will be deformed to the extent that the models cannot fit. Accordingly, the wide-angle X-ray diffraction pattern reflects only the local nematic character. It is important to investigate the structure that is possibly responsible for the cholesteric torsion.

As judged from the pattern of I,[13-15] the molecular packing was not simple. The local structure is positionally disordered. As an indication for a certain fundamental structure, film I formed a stoichiometric complex (C) with methanol characterized by sharp reflections.[7,8] The transformation from I to C was caused instantaneously by immersing the film into methanol, as was the reverse by drying the films. Continuous swelling between I and C was observed by immersing the film in mixtures of methanol and water.[8] The red shift of the cholesteric pitch was linearly related to the lattice swelling. This may support the cholesteric torsion between molecular layers.

In contrast, the PMLG film (II) prepared from the solution in chloroform was free from the twisted structure and showed a well-defined diffraction pattern.[16-18] Neither the optical properties characteristic of the cholesteric torsion nor the complex formation with methanol was observed for II. The structure is hexagonal with a lateral unit-cell dimension of a = 11.95 Å. The simple molecular packing does not suggest any particular arrangement of the up- and down-pointing helices. Worth noting is that the apparent high crystallinity of II is a result of the random arrangement of up and down helices in the hexagonal lattice points.[19] Because of this closest packing, there may be no room to incorporate methanol molecules.

The structures of films I and II may be distinguished from each other by the manner of arrangement of up and down helices. If it were essentially the same, the transition between I and II should be caused easily by heat treatment, since the side-chain motion takes place above 0 °C and relaxes the structure. However, the very slow transition from II to I was observed by the exhaustive heat treatment above 200 °C over several hours.[7] This may be due to the fact that to rearrange up and down helices requires much time even at high temperature. Film II exhibited a large mechanical relaxation at ca. 180 °C, while the relaxation of I was suppressed in this temperature range.[13-15] These suggest that in I the helices are assembled with a particular

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interaction between up and down helices.

The X-ray diffraction technique is useful only for structures showing well-defined patterns. About 10% disorder in interchain distances is enough to deteriorate the pattern. Biological systems composed of fibrous proteins often assume supermolecular structures, where such kind of disorder may not be important for their functions. The broad pattern does not imply that the structure is amorphous. From this point of view, the structures of PMLG film I and the complex with methanol (C) are very interesting. Since the diffraction data were not sufficient, some speculations were inevitable. The object of this work is to analyze the fundamental structure underlying films I and C without calling minor ambiguities into question.

Experimental Section

The PMLG sample with molecular weight of 11×10^4 was kindly supplied by Ajinomoto Co. Inc., Japan. The film was prepared on a glass plate by casting the solution in DCE at room temperature. In order to obtain films with a good extent of uniplanar orientation, the evaporation of solvent was restricted to be slow. The film was stripped from the plate by immersing it in methanol and subsquently dried in vacuo.

Wide-angle X-ray diffraction patterns were taken with Ni-filtered Cu Kα radiation by using a flat-plate camera. The specimens were cut into strips with dimensions about $10 \times 0.3 \times 0.3$ mm and mounted on the sample holder with the film surface parallel (EV) and perpendicular (TV) to the incident beam. The specimen of the complex was prepared by sealing the film in a quartz capillary tube with methanol. The reflection profiles were recorded by scanning the EV and TV photographs with a microphotometer in various directions. The intensities were integrated in reciprocal space by taking into account the type of uniplanar orientation and corrected for Lorentz–polarization factors.

Analytical. The crystal structure factors of equatorial reflections of C were calculated by

$$F(hk0) = F_\alpha(R)\sum_j \exp\{2\pi i(hx_j + ky_j)\} \quad (1)$$

where $F_\alpha(R)$ is the molecular structure factor of the helix scaled per amino acid residue; x_j and y_j are the fractional coordinates of the position of the jth helix. The contribution from the methanol molecules was neglected at first. $F_\alpha(R)$ was calculated for the standard 18-residue five-turn α-helix by

$$F_\alpha(R) = \sum_k f_k J_0(2\pi R r_k) \exp\{-B_k(R/2)^2\} \quad (2)$$

where R is the cylindrical radial coordinate of the $hk0$ reciprocal-lattice point; J_0 is the zeroth-order Bessel function; f_k, r_k, and B_k are the atomic scattering factor, the cylindrical radial coordinate, and the isotropic temperature factor of the kth atom in a residue, respectively. Since the F_α is to be scaled per residue, the crystal structure factors are given as the values per unit cell divided by a factor of 18. The side chains are considered to be fairly disordered, but they are not negligible. Since F_α depends only on the atomic radial coordinates, the following two side-chain conformations, extended and contracted, were considered:

	N—C$^\alpha$—C$^\beta$—C$^\gamma$—CO—O—C			
	χ_1	χ_2	χ_3	χ_4
model 1	T	T	T	T
model 2	$\bar{G}$	T	G	T

where T and G ($\bar{G}$) are trans and gauche conformations, respectively. A value of $B = 5$ Å^2 was assumed for the backbone atoms, and larger values (≤ 30 Å^2) were employed for the outer atoms. The molecular structure factors calculated for the two models (including the hydrogen atoms) were simply averaged as F_α.

The crystal structure factors were finally calculated by taking into account the contribution from the methanol molecules. The molecular structure factor was approximated by

$$F_M(R) = 18 \exp\{-(B_f + B_r + B_g)(R/2)^2\} \quad (3)$$

The effect of decay of the atomic scattering factors with R was expressed by $B_f \sim 15$ Å^2. A factor $\sin(2\pi R r_0)/2\pi R r_0$ for a rotating or randomly oriented molecule was expressed with $B_r \sim 30$ Å^2, where r_0 is the length of the C–O bond. The effect of the translational motion or the uncertainty in the position of the center of gravity is expressed by $B_g = 8\pi\sigma^2$, where σ^2 is the mean-square amplitude. Finally, we assumed a value of $B_f + B_r + B_g = 200$ Å^2, corresponding to $\bar{\sigma} = 1.4$ Å.

The electron-density projection along the c axis is determined by Fourier synthesis in the equatorial plane. The two-dimensional structure of the hexagonal system (corresponding to complex C) is probably centrosymmetric and has dyad axes (space group: $p6mm$), where the $F(hk0)$'s are real and $F(hk0) = F(kh0)$. Since the amplitudes ($|F_o|$ on a relative scale) are obtained from the intensity data, the projection can be calculated by assigning the phase factors (+1 or −1) to them. The electron density projected on the point (x, y) was defined here by the average of the density at (x, y, z) along the z axis over a repeat distance of $c = 27.0$ Å (assumed for the standard α-helix):

$$\rho(xy) \equiv \frac{1}{c}\int_{z=0}^{c} \rho(xyz)\, dz = \frac{18}{V}\sum_h \sum_k F_o(hk0) \cos\{2\pi(hx + ky)\} \quad (4)$$

where V is the unit-cell volume.

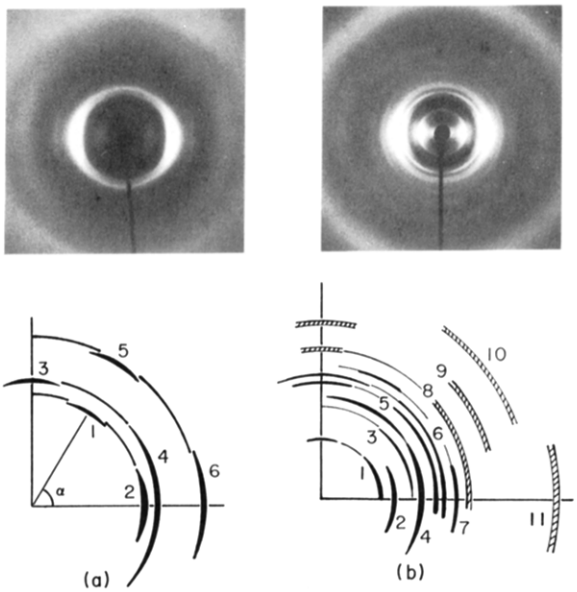

Figure 1. X-ray EV pattern of PMLG film prepared from the solution of DCE and the schematic diagram of the first quadrant of the pattern: (a) as-cast film I; (b) the complex with methanol (C). The film surface is vertical.

Results and Discussion

X-ray Data and Unit Cells. The EV diffracton patterns and their schematic diagrams of I and C are shown in Figure 1. Since the film surface was set to be vertical, the direction of the cholesteric axis was horizontal. The positions of intensity maxima are denoted below by the angle α measured from the horizontal line.

For I, only six reflections were recognized, and all of them were considered to be equatorial. In spite of the limited data, the spacings (Table I) could not be explained by any primitive molecular packing. Strong reflection no. 2 at $\alpha \sim 0°$, for instance, may express the existence of molecular layers parallel to the film surface with a spacing of 11.9 Å. Reflection no. 3 at $\alpha \sim 90°$ does not look strong on the pattern, but the integrated intensity is strong as it is multiplied by $\sin(\alpha)$. Its spacing, 10.8 Å, may correspond

Table I
X-ray Data of PMLG Film I and the Complex with Methanol (C)

no.	α,[a] deg	intens[b]	d_{obs}, Å	d_{calc}, Å	indices hkl
			Film I		
1	60	w	12.2	12.3	300, $\bar{3}30$
2	0	vs	11.9	12.0	030
3	90	m	10.8	10.8	$4\bar{2}0$
4	0–30	s	10.6	10.5	220, $\bar{2}40$
5	60	vw	8.1	8.0	410, $\bar{4}50$
6	~0	m	7.8	7.9	140, $\bar{1}50$
			Complex C		
1	30, 90	m	22.9	22.8	110
2	0	w	19.0?	19.7	200
3	30, 90	w	14.6	14.9	210
4	0, 60	s	13.2	13.2	300
5	0–30	m	11.4	11.4	220
6	0	m	10.9	10.9_5	310
7	0	w	9.9	9.9	400
8	0–30	w	9.0	9.0_6	320
9	~0	vw	7.7	7.6	330
10	~40	vw	6.4	6.5	430
11	~0	vw	5.6	5.6	700

[a] α is the angle indicating the position of the intensity maximum in the EV pattern measured from the direction normal to the film surface. [b] vs, very strong; s, strong; m, moderate; w, weak; vw, very weak.

Table II
Lattice Parameters of PMLG Films

parameter	I	C
lattice constants[a]		
a/Å	43.2	45.6
b/Å	42.0	45.6
c/Å	27.0	27.0
γ/deg	121	120
N[b] (α-helix)	12.6	12.6
Z (methanol)		~100
density/(g cm^{-3})		
calcd	1.29	~1.21
obsd	1.29	

[a] The dimension of c was assumed for the standard α-helix. [b] N is the statistical-average number of helices passing through the unit cell.

to the distance between molecules in the layer. However, this model could not explain the spacings of other reflections, and the density calculated by assuming a standard unit height of 1.5 Å (ρ_c = 1.23 g cm^{-3}) was much smaller than the observed value (ρ_0 = 1.29 g cm^{-3}). Taking into account the features of the pattern and the density, we examined various models, but all attempts failed.

The complex with methanol (C) showed sharp reflections (Figure 1b), some of which represented large spacings that could not be explained by simple molecular packings. Since the transformation between I and C was instantaneous and reversible, the structures are essentially isomorphous (not crystallographically). In the EV pattern of C, 11 reflections were observed (Table I). Strong reflection no. 4 appeared at $\alpha \sim 0$ and 60°. The spacings and the integrated intensities (corrected for multiplicity) were the same for the arcs at $\alpha \sim 0$ and 60°. The structure was determined to be hexagonal with the lateral unit-cell dimension of a = 45.6 Å (Tables I and II). The spacing of reflection no. 2 differed slightly from the calculated value. Since its intensity was very weak, the results were not altered without it.

By referring to the structure of C, we determined the unit cell of I to be pseudohexagonal with dimensions listed in Table II. The uniplanar orientation in I and C was of the type with the a^* axis perpendicular to the film surface.

From the hexagonal symmetry, the number N of the helices passing through the unit cell of C is 12 or 13. The same number of helices will pass through the unit cell of I. From the observed value of the density, N is expected to be 12.6. As discussed below, this fractional number was interpreted as a statistical average in this work. By assuming the additivity of specific volume, the number of methanol molecules, Z, contained in the unit cell was estimated to be 100, corresponding to 0.4–0.5 molecule per amino acid residue.

Table III
Calculated (F_c) and Observed ($|F_o|$) Structure Factors of PMLG Complex with Methanol (C)

hkl	F_α[a]	F_M[b]	F_c	$\|F_o\|$
100	66.2	17.4	0.8	
110	51.0	16.3	21.3	44
200	44.8	15.8	-18.1	15
210	30.3	14.4	-29.8	29
300	23.5	13.5	-107.9	96
220	16.2	12.3	53.0	71
310	14.4	11.9	71.3	55
400	10.6	10.8	-44.3	37
320	8.2	9.8	24.7	40
410	7.4	9.2	5.0	
500	6.3	8.1	-11.6	
330	6.0	7.6	38.5	40
420	5.9	7.3	1.7	
510	5.7	6.7	3.6	
600	5.4	5.7	-10.9	
430	5.3	5.5	-23.3	20
520	5.2	5.2	-7.6	
610	4.6	4.5	-6.9	
440	3.8	3.9	11.9	
700	3.6	3.7	66.4	65
530	3.6	3.7	0.0	

[a] F_α is the molecular structure factor of the α-helical PMLG chain per amino acid residue. [b] F_M is the molecular structure factor of methanol.

Equatorial Fourier Synthesis. The crystal structure factors were calculated for various two-dimensional models of C by using eq 1, where the methanol molecules were first neglected. By trial and error, however, we could not find any reasonable structure. Then the electron-density equatorial projection was calculated according to eq 4 by assuming the space group $p6mm$. If we use eq 4 for the structure that is not of $p6mm$, the number and the values of density maxima will appear unreasonable.

The $\rho(xy)$ maps were calculated from the observed $|F_o|$ data with all combinations of the phase factors. Only one of them turned out reasonable. Then the helices were placed in the positions of density maxima, and the crystal structure factors were calculated again. The scale factor and the phase factors were finally determined by refining the structure with alternate calculations of $\rho(xy)$ and $F(hk0)$. For the calculation of $\rho(xy)$, $F(000) = \sum f_k = NF_\alpha(0) + ZF_M(0)/18 \sim 1060$ was included.

In the unit cell of the map (Figure 2), 13 major peaks are observed, where three of them are independent. For the complete α-helix, the maximum density ρ_{max} is known to be ~1.0 electrons Å^{-3} for the core region.[19-21] The positions and the ρ_{max} (in electrons Å^{-3}) of the three independent helices are

Wyckoff position	x	y	ρ_{max}
1a	0	0	0.75
6e	0.28	0.14	1.0
6e	0.57	0.14	0.9

The small value of ρ_{max} = 0.75 for the helix at Wyckoff position 1a was judged to be out of the experimental error. Therefore, this position was considered to be occupied with

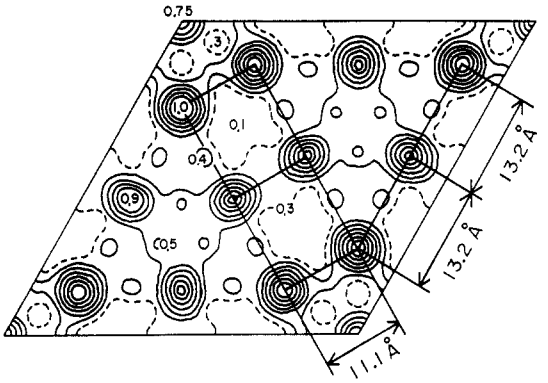

Figure 2. Equatorial electron-density projection of PMLG complex with methanol (C). The figures indicate the density in units of electrons Å^{-3}. Contours are at intervals of 0.1 electron Å^{-3}.

Figure 3. Structure amplitudes of PMLG complex with methanol (C): (—) observed; (···) calculated with only PMLG chains; (- - -) calculated with PMLG and methanol molecules.

a probability ~0.6. The vacancies (with a probability ~0.4) will be filled by the methanol molecules. The statistical distribution of the vacancy may explain the disordered molecular packing in film I. These support the fractional number of $N = 12.6$.

To confirm the *p6mm* space group, we refined the structure by releasing the conditions constrained by the symmetry. However, all models were reduced to the above-mentioned structure.

In the electron-density map (Figure 2), two kinds of interchain distances appear: one is 13.2 Å and the other is 11.1 Å. Small peaks are observed between the major peaks of the helices separated by 13.2 Å. These may correspond to the methanol molecules. There are 18 such positions in the unit cell. If the methanol molecules are closely packed as spheres of a diameter 5 Å in each column, the number of methanol molecules Z will be ca. 97. This is consistent with the estimation. In accord with the hexagonal symmetry, 108 molecules were included as the 4.5-Å spheres. The calculated structure factors (F_c) are compared with the observed data in Table III. As is shown in Figure 3, the agreement between $|F_c|$ and $|F_o|$ was much improved by taking into account the contribution of the methanol molecules. The molecular structure factors of the helix (F_α) and methanol (F_M) are also given in Table III. The discrepancy factor $\sum||F_c| - |F_o||/\sum|F_o|$ was 0.20 for the 11 observed reflections and 0.32 for all reflections. The agreement was fairly good for the data including the missing reflections.

Molecular Association. The two-dimensional structure of C is illustrated in Figure 4a. The short interchain distance 11.1 Å is considerably smaller than the 12-Å distance in II. Aside from the helices at 1a, the 11.1-Å distance may be assigned to that between pairs of antiparallel helices, while the long interchain distance 13.2 Å may be assigned to that between pairs of parallel helices. The reverse assignment causes contradictions.

In view of the structure, it is speculated that the fundamental unit formed in the liquid crystal in DCE is

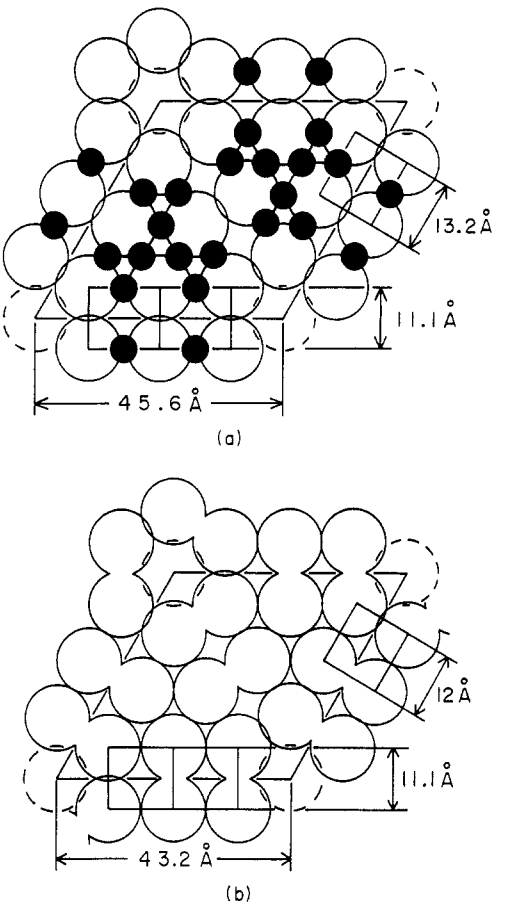

Figure 4. Two-dimensional molecular arrangement in PMLG film prepared from the solution in DCE: (a) complex with methanol (C); (b) a model of molecular association in as-cast film I. The film surface is horizontal. Large circles, α-helices; dashed circles, α-helices existing with a probability of ~0.6; small black circles, methanol molecules.

the antiparallel bimolecular association. A model of packing consistent with the hexagonal symmetry is illustrated in Figure 4b, where the six helices (located in half the unit cell) are assembled to be directed in the same way. Such assembly may be stabilized in the liquid crystal by the incorporated solvent molecules, which are replaced by the methanol molecules in C. The 1a positions may be left for the solvent molecules or filled by the isolated helices.

After the methanol molecules are evacuated, the interchain spacings of 13.2 Å may be reduced to ca. 12 Å (the spacing in II), while the short spacings of 11.1 Å will be no longer altered. By this contraction, the unit-cell dimension is expected to decrease to ca. 43.2 Å, which is very close to the observation. The structure may not be stable, since the packing seems uncomfortable (not closest as in II) and the electrostatic interactions are unfavorable. The helices at positions 1a (shown by dashed circles in Figure 4) are surrounded tightly by three parallel and three antiparallel helices. If positions 1a are statistically vacant, the structure is to be highly stressed. This may elucidate the disordered structure of I.

The association between up and down helices separated by 11.1 Å is probably very strong. This may be a reason for the slow transition from I to II caused by heat treatment. Furthermore, the main-chain motions responsible for the mechanical relaxation will be suppressed. In spite of the disordered structure, the relaxation of I was actually observed at much higher temperature than the crystalline relaxation observed at ca. 180 °C for II.[13-15]

Highly assembled supermolecular structures have not

been observed for ordinary polymers. Synthetic polypeptides are extraordinary and functional to yield a supermolecular structure like biological polymers. No direct information could be derived for the relation between the molecular assembly and the cholesteric twisted structure. It may not be fruitful to speculate about it at present. The colored film of PBLG showed a characteristic diffraction pattern, the features of which were different from those of PMLG film but indicative of some helix assembly.[9,22-25] The PBLG film formed a complex with DMF or benzyl alcohol.[26-28] The analysis will be reported in a forthcoming paper.

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