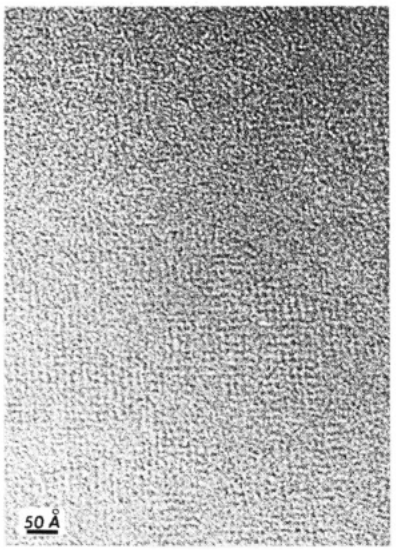

Figure 6. Image of crystallites formed by heating $(SiPcO)_n$.

$(HO(SiPcO)_nH$ or $SiPc(OH)_2)$, sublimation of the species formed, and, finally, repolymerization of the sublimed species. It could also involve the thermal depolymerization of the chains to SiPc=O and sublimation and repolymerization of this species. If one or more processes like these are involved, the low efficiency of the film formation process and the thinness of the films are understandable.

Additional Studies. Preliminary work has yielded images of films formed by heating the cofacial naphthalocyanine polymer $(SiNcO)_n$[26] and collecting the condensable vapors on KCl. These images show that the films are composed of platy crystallites lying horizontally. They indicate that the crystallites contain vertical ring stacks and that the rings are parallel to the film plane. The unit cell of the crystallites is apparently tetragonal. On the basis of the data available, it is thought that these films may be composed of crystallites of $(SiNcO)_n$ in which the molecules are oriented vertically.

Summary. Images of films of $(AlPcF)_n$ formed by vapor deposition on KCl show crystallites in which the molecules are oriented vertically. A photographically averaged image of one of the crystallites shows that its rings are eclipsed.

Acknowledgment. We thank Mr. R. A. Camps of the High-Resolution Electron Microscope facility of the Department of Metallurgy of Cambridge University for his cooperation and assistance. We also thank the Office of Naval Research and the Science and Engineering Research Council for support of this work.

Registry No. $(AlPcF)_n$, 74018-71-6; $(SiPcO)_n$, 39114-20-0.

References and Notes

(1) Joyner, R. D.; Kenney, M. E. *J. Am. Chem. Soc.* **1960**, *82*, 5790.
(2) Joyner, R. D.; Kenney, M. E. *Inorg. Chem.* **1962**, *1*, 717.
(3) Kroenke, W. J.; Sutton, L. E.; Joyner, R. D.; Kenney, M. E. *Inorg. Chem.* **1963**, *2*, 1064.
(4) Shorin, V. A.; Meshkova, G. N.; Vartanyan, A. T.; Pribytkova, N. N.; Al'yanov, M. I.; Brodkin, V. F. *Izv. Vyssh. Uchebn. Zaved., Khim. Khim. Tekhnol.* **1973**, *16*, 1904; *Chem. Abstr.* **1974**, *80*, 97325x.
(5) Linsky, J. P.; Paul, T. R.; Nohr, R. S.; Kenney, M. E. *Inorg. Chem.* **1980**, *19*, 3131.
(6) Kusnesof, P. M.; Nohr, R. S.; Wynne, K. J.; Kenney, M. E. *J. Macromol. Sci., Chem.* **1981**, *A16*, 299.
(7) Fischer, K.; Hanack, M. *Chem. Ber.* **1983**, *116*, 1860.
(8) The conditions used to acquire the electron microscopy data given in this paper lead to uncertainties in the interpretation of these microscopy data.
(9) Shimura, M.; Toyoda, A. *Jpn. J. Appl. Phys., Part I* **1984**, *23*, 1462.
(10) Wynne, K. J. *Inorg. Chem.* **1985**, *24*, 1339.
(11) Dirk, C. W.; Inabe, T.; Schoch, K. F., Jr.; Marks, T. J. *J. Am. Chem. Soc.* **1983**, *105*, 1539.
(12) Electron microscopy data on $(SiPcO)_n$[13,14] have been interpreted as indicating parallel packing of the chains, but again the conditions used to acquire the data lead to uncertainties in their interpretation.
(13) Linsky, J. P. Ph.D. Thesis, Case Western Reserve University, Cleveland, OH, 1970.
(14) Schechtman, L. A.; Kenney, M. E. *Proc.-Electrochem. Soc.* **1983**, *83-3*, 340.
(15) Zhow, X.; Marks, T. J.; Carr, S. H. *Mol. Cryst. Liq. Cryst.* **1985**, *118*, 357.
(16) Zhow, X.; Marks, T. J.; Carr, S. H. *Polym. Mater. Sci. Eng.* **1984**, *51*, 651.
(17) Nohr, R. S.; Kusnesof, P. M.; Wynne, K. J.; Kenney, M. E.; Siebenman, P. G. *J. Am. Chem. Soc.* **1981**, *103*, 4371.
(18) Diel, B. N.; Inabe, T.; Lyding, J. W.; Schoch, K. F., Jr.; Kannewurf, C. R.; Marks, T. J. *J. Am. Chem. Soc.* **1983**, *105*, 1551.
(19) Owen, J. E.; Kenney, M. E. *Inorg. Chem.* **1962**, *1*, 334.
(20) Preliminary reports of this work have appeared.[21,22]
(21) Fryer, J. R.; Kenney, M. E. *Inst. Phys. Conf. Ser.* **1985**, *78*, 441.
(22) Fryer, J. R. *Mol. Cryst. Liq. Cryst.* **1986**, *137*, 49.
(23) Fryer, J. R.; Holland, F. *Proc. R. Soc. London, A* **1984**, *393*, 353.
(24) Because images with partially developed quatrefoils had not been obtained at the time of the first report,[21] it was incorrectly concluded that the rings are staggered.
(25) Gaudiello, J. G.; Almeida, M.; Marks, T. J.; McCarthy, W. J.; Butler, J. C.; Kannewurf, C. R. *J. Phys. Chem.* **1986**, *90*, 4917.
(26) Moyer, T. J.; Schechtman, L. A.; Kenney, M. E. *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **1984**, *25*, 234.

Notes

Crystallization in Polyproline and Gelatin Blends by X-ray Diffraction of Stretched Films

S. A. FOORD and E. D. T. ATKINS*

H. H. Wills Physics Laboratory, University of Bristol, Tyndall Avenue, Bristol BS8 1TL, U.K.
Received May 18, 1987;
Revised Manuscript Received August 10, 1987

Introduction

From X-ray diffraction two solid-state conformations I and II have been found for polyproline. Form I[1] is a right-handed helix having $3^1/_3$ residues per turn with all cis peptide bonds while form II[2,3] is a left-handed 3-fold helix with all trans peptide bonds.

Gelatins may be regarded as water-soluble denaturation products from collagen.[4] The native collagen molecule contains three polypeptide α-chains each about 1000 amino acids long and with a triplet repeat scheme of [glycine-X-Y]$_n$ where X is frequently proline and Y hydroxyproline.[5] The three α-chains are thought to have a conformation based on the polyproline II structure but are further coiled into a right-handed triple-helix structure.[6] A given gelatin contains a characterizing spectrum of collagen molecule parts which may include whole α-chains (α-gelatin), co-

0024-9297/88/2221-0262$01.50/0

Table I
Solution Preparation Details for the Films X-rayed in Figure 1

	net polymer, mg/mL	% poly-proline	hours at 50 °C	days at room temp prior to casting
Figure 1a	10	0%	3	0
Figure 1b	7	15%	3	0
Figure 1c	$6^1/_4$	35%	$2^1/_2$	0
Figure 1d	$4^1/_2$	50%	17	0
Figure 1e	5	60%	4	0
Figure 1f	$6^1/_4$	60%	4	4

valently linked α-dimers (β-gelatin), α-trimers (γ-gelatin), and higher multiplets and various fragments according to the collagen tissue source and severity of the extraction process.[4]

In a hot solution above the melting point of collagen the gelatin molecules exist in random coil form. On cooling below 40 °C gelation can occur, resulting in a fibrillar network, as observed qualitatively by electron microscopy,[7-10] and containing a proportion of renatured collagen-like structure as indicated by X-ray diffraction[11] and other studies.[12] During renaturation the α-chains are expected at some stage to adopt a conformation similar to polyproline II and it might be anticipated that in a mix with polyproline, itself adopting the type II structure, mutual poisoning of crystallization processes could occur. However, polyproline II cannot be coiled with α-chains in a collagen triple helix structure, as glycine at every third in the α-repeat is the only amino acid with a side group compact enough to occupy the interior of the molecule.[6] It is of interest to see if mutual poisoning or segregation occurs in mixes of the two polymers.

Experimental Section

Materials. Polyproline (lot 124F-5016, Sigma Chemical Co.), with molecular weight >30 000 by viscosity determination,[13] was used. The gelatin was also from Sigma (lot 96C-0306), extracted from swine skin and having a bloom number of about 300. SDS electrophoresis on 55% acrylamide gel showed the gelatin to have a molecular weight continuum ranging from less than 30 000 to more than 300 000.

On the basis of its solubility behavior, polyproline was found to be form II which dissolves easily in cold water but becomes less soluble when hot[14,15] and precipitates out at ~65 °C. To avoid precipitation problems it was decided to use 0.5 M (2.9%) acetic acid as solvent. The two components were weighed on an Oertling microbalance, placed in a test tube, 2.5 mL of solvent were added, chilled to dissolve the polyproline and then heated to 50 °C for at least 2 h to dissociate the gelatin, and the solution was allowed to dry on Teflon at room conditions. About 5–7 mg/mL of net polymer in solution was found to result in dried films with adequate mechanical properties for stretching and with sufficient scattering power for X-ray exposures. Films with more than ~70% polyproline were mechanically unviable for the stretching process. Samples were stretched under tension at high relative humidity.

X-ray patterns, as shown in Figure 1, were obtained by using an evacuated box camera and nickel-filtered pinhole-collimated Cu Kα radiation. Patterns were calibrated from calcite (0.3035 nm) dusted onto the samples. Solution preparation details are summarized in Table I.

Results, Analysis, and Discussion

Figure 1a is a typical gelatin pattern showing the collagen wide-angle pattern[6] with a clear equatorial reflection at 1.05 nm and the characteristic 0.3, 0.4, 1.0 nm layer lines. A corresponding gelatin in water pattern looked much the same though was twice as strong per unit mass of gelatin.

Figure 1b shows the effect of mixing 15% polyproline with gelatin. The gelatin pattern is present after orientation but so are additional smooth rings characteristic of polyproline II[16] (see Table II). The polyproline crystals are thus randomly oriented within the gelatin matrix.

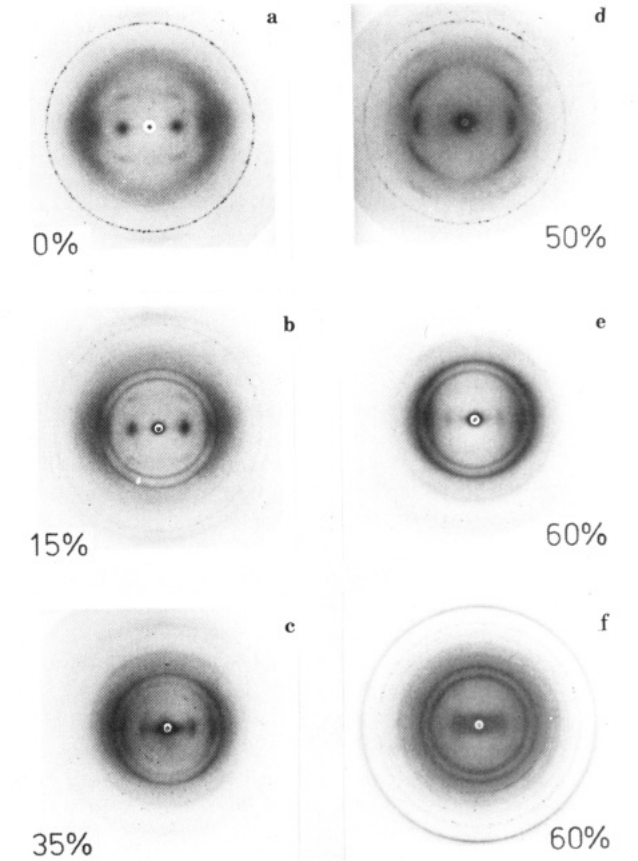

Figure 1. X-ray diffraction patterns obtained from stretched films of gelatin/polyproline mixtures. The stretch direction is vertical and the "spotted" ring is a calcite calibration. Percentage of amounts of polyproline added to gelatin are indicated. Relative intensities may be distorted in the reproduction process.

Table II
Polyproline II Spacings in Nanometers from Figure 1b Compared with the Spacings of the Ring Pattern in Figure 1f

Figure 1b, nm	Figure 1f, nm	Figure 1b, nm	Figure 1f, nm
0.578	0.592	0.364	0.365
0.490	0.501		0.333
	0.430		0.323
	0.386	0.281	0.271

Parts c and e of Figure 1 are similar in nature and are from 35% and 60% polyproline mixes, respectively. In these patterns the gelatin signal is greatly diminished with only the 1.05-nm equatorial visible to confirm the gelatin chains are oriented. The stronger polyproline II signal comes from a population of crystals some of which are preferentially oriented with the chain axis parallel to the gelatin chains as judged from the equatorial intensification of the 0.578-nm ring.

Figure 1d is from a 50% polyproline mix where the blend was allowed to stand for 17 h at 50 °C in 0.5 M acetic acid prior to casting. The pattern is almost entirely from oriented polyproline II crystals with very faint traces of the gelatin 1.05-nm equatorial. There are two possible reasons why the polyproline signature is so clear in this pattern, both having to do with the long standing period. First, the gelatin is progressively broken down in molecular weight with time during the treatment. For instance a gelatin solution, without polyproline, may eventually be

so degraded that the dried film is brittle with no pliability for stretching. At the end of the 17-h period the gelatin is not degraded to that extent (polyproline does not produce pliable films on its own) but its molecular weight spectrum may be sufficiently changed for it to be regarded as a different gelatin for the purposes of the experiment. Second, the stand at elevated temperature may have promoted growth of polyproline crystals due to a decrease in solubility at high temperatures. This pattern indicates that gelatins could be a useful matrix material for obtaining oriented fiber patterns from polypeptides in general. Note that the gelatin could be added as a cool solution to prepared crystals in solution and that acidic conditions are not necessary for matrix formation.

Figure 1f comes from a 60% polyproline mix subjected to the same treatment (including stretching) as that which gave Figure 1e but the solution was allowed to stand at room temperature for 4 days before casting. The pattern shows four diffuse rings, matching with slightly modified spacings to the polyproline II rings in Figure 1b. In addition there are some other weak rings and the whole system, see Table II, does not correspond to any of the paste patterns observed by Sasisekharan.[16] The pattern also shows a pronounced equatorial streak ending in diffuse equatorial reflections at about 1.1 nm which may come from the gelatin. It seems likely that this pattern is of a previously unreported polyproline crystal form.

The suppression of the gelatin pattern in the mixes is an interesting phenomenon. Despite disappearance of the pattern, the gelatin matrix still lends mechanical integrity to the films, enabling them to be stretched with the plasticizing water vapor.

To analyze Figure 1a–f for the effect of polyproline on collagen renature (whether this occurs mostly in solution followed by aggregation of the renatured molecules[17-21] or during mechanical stretching of the films[11,12]) it is necessary to relate the strength of reflections, for instance the 1.05-nm equatorial,[11] to collagen content.

Aside from the 0.286-nm meridional, collagen/gelatin reflections have strengths which could be influenced by the nature of the molecular packing as well as the extent of renature. Quantitative data to show if relative reflection strengths do vary with sample treatments are not currently available.

The 0.286-nm reflection from a periodicity along renatured collagen molecules is too weak in the given patterns to be useful, but the 1.05-nm equatorial is clear in most of them. Thus the packing effects can be investigated through the 1.05-nm reflection.

The quantity of gelatin pattern predicted from a thin stretched sample on the basis of gelatin scattering mass in the beam is easily estimated assuming pattern attenuation by the polyproline phase is both slight (since the majority of X-ray photons pass through the sample unaffected by either phase) and about equal to that by gelatin itself. Pattern strength should then be proportional to gelatin concentration multiplied by net X-ray flux divided by sample extension. Allowing as well for the different print times of the photographs in the plate, these should respectively show 1, 1, 0.8, 1.5, 0.5, 0.25 units of gelatin pattern. The unit of gelatin pattern is standardized in terms of Figure 1a on the basis of the number of exposed film grains. The number of exposed film grains in the print is taken as being linearly proportional (where saturation has not occurred) to the number of X-ray photons photographically captured. Against this scale Figure 1c, for instance, should contain in features of its gelatin pattern 0.5 of the grains at equivalent features of Figure 1a. Compare, for example, the prominent equatorial signal at a spacing of 1.05 nm.

By inspection it is possible to appreciate the 1.05-nm equatorial reflection falls off quicker with increasing polyproline content than predicted above. This indicates that the two polymers do not totally segregate and the polyproline poisons gelatin packing to a degree. Analogously, low molecular weight fractions in a pure gelatin might also interfere with packing and modify equatorial and other nonmeridional signals. The latter reflections are thus potentially unreliable indicators of renaturation content.

Acknowledgment. We thank the Agriculture and Food Research Council for supporting this work and the Institute of Food Research, Bristol, for analyzing the gelatin.

Registry No. Polyproline (homopolymer), 25191-13-3; polyproline (SRU), 25213-33-6.

References and Notes

(1) Traub, W.; Shmueli, U. *Nature (London)* **1963**, *198*, 1165–1166.
(2) Cowan, P. M.; McGavin, S. *Nature (London)* **1955**, *176*, 501–503.
(3) Sasisekharan, V. *Acta Crystallogr.* **1959**, *12*, 897–903.
(4) Johns, P.; Courts, A. In *A Science and Technology of Gelatin*; Ward, A. G., Courts, A. Academic Press: London, 1977; pp 137–177.
(5) Glanville, R. W.; Kuhn, K. In *Fibrous Proteins; Scientific, Industrial and Medical Aspects*; Parry, D. A. D., Creamer, L. K. Academic Press: London, 1979; Vol. 1, 133–150.
(6) Ramachandran, G. N. In *Treatise on Collagen, volume 1 Chemistry of Collagen*; Ramachandran, G. N., Ed., Academic: London & New York, 1967; pp 103–183.
(7) Titova, Y. F.; Belavtseva, Y. M. *Biophysics* **1984**, *29*, No. 2, 372–374.
(8) Mikhailov, A. N.; Titova, Y. F.; Belavtseva, Y. M.; *Biophysics* **1980**, *24*, 450–455.
(9) Tomka, I.; Bohonek, J.; Spühler, A.; Ribeaud, M. *J. Phot. Sci.* **1975**, *23*, 97–103.
(10) Titova, E. F.; Belavtseva, E. M.; Braudo, E. E.; Tolstoguzov, V. B. *Colloid Polymer Sci.* **1974**, *252*, 497–503.
(11) Tanioka, A.; Miyasaka, K.; Ishikawa, K. *Biopolymers* **1976**, *15*, 1505–1511.
(12) Galatik, A.; Blazej, A. *Collect. Czech. Chem. Commun.*, **1978**, *45*, 628–40.
(13) Mattice, W. L.; Mandelkern, L. *J. Am. Chem. Soc.* **1971**, *93*, 1769–1777.
(14) Mattice, W. L.; Mandelkern, L. *Macromolecules* **1971**, *4*, No. 3, 271–274.
(15) Ciferri, A.; Orofino, T. A. *J. Phys. Chem.* **1966**, *70*, 3277–3285.
(16) Sasisekharan, V. *J. Polym. Sci.* **1960**, *47*, 373–390.
(17) Thorn, I.; Eagland, D. *Biopolymers* **1984**, *23*, 353–361.
(18) Eagland, D.; Pilling, G. *Biopolymers* **1980**, *19*, 147–164.
(19) Finer, E. G.; Franks, F.; Phillips, M. C.; Sugget, A. *Biopolymers* **1975**, *14*, pp 1995–2005.
(20) Eagland, D.; Pilling, G.; Wheeler, R. G., *Discuss. Faraday Soc.* **1974** No. 57, 181–209.
(21) Harrington, W. F.; Rao, N. V. *Biochemistry* **1970**, *9*, 3714–3724.

Marker Retention in Inverse Gas Chromatography Experiments on Polymers

MOHAMMAD J. EL-HIBRI and PETR MUNK*

Department of Chemistry and Center for Polymer Research, The University of Texas at Austin, Austin, Texas 78712.
Received May 13, 1987

Introduction

In gas–liquid chromatography, an ideal marker is a substance which has no retention on the stationary phase of the column. While any real marker must have a nonzero retention, in standard GC application a marker is con-

0024-9297/88/2221-0264$01.50/0