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Polymers of Tripeptides as Collagen Models
VIII. X-ray Studies of Four Polyhexapeptides

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The structures of four polyhexapeptide collagen models have been investigated by X-ray diffraction. It has been found that $(Gly\text{-}Ala\text{-}Pro\text{-}Gly\text{-}Pro\text{-}Pro)_n$, $(Gly\text{-}Pro\text{-}Ala\text{-}Gly\text{-}Pro\text{-}Pro)_n$, $(Gly\text{-}Ala\text{-}Pro\text{-}Gly\text{-}Pro\text{-}Ala)_n$ and $(Gly\text{-}Ala\text{-}Ala\text{-}Gly\text{-}Pro\text{-}Pro)_n$ all show typical collagen-like diffraction patterns, although there is some variation among the polymers in modes of lateral packing and in axial repeat distances. A systematic investigation of sterically allowed collagen-like structures has been carried out for each of the four polyhexapeptides. The intensity ratios of the equatorial reflections were calculated for the various sterically possible structures and compared with the observed values. In this way, it has been shown that none of the polyhexapeptides adopts the conformation of the two-bonded model for collagen, even though, from their amino acid sequences, three of the polymers might be expected to do so. On the other hand, for all four polyhexapeptides, the analyses have revealed sterically acceptable one-bonded structures which are consistent with the intensity data. These structures all closely resemble that found for $(Pro\text{-}Gly\text{-}Pro)_n$, as described in Yonath & Traub (1969). It is suggested that collagen itself probably has the same one-bonded conformation as these synthetic model compounds.

1. Introduction

Yonath & Traub (1969) give a description of the detailed structure analysis of $(Pro\text{-}Gly\text{-}Pro)_n$. The polymer is shown to have a collagen-like triple-helical structure and to resemble the collagen II model (Rich & Crick, 1961; Cowan, McGavin & North, 1955) in its mode of interchain hydrogen bonding.

Several other polytripeptides of the type $(Gly\text{-}Pro\text{-}X)_n$, where X is an amino or imino acid residue, have been found to resemble collagen in their X-ray patterns. These include $(Gly\text{-}Pro\text{-}Hypro)_n$ (Rogulenkova, Millionova & Andreeva, 1964), $(Gly\text{-}Pro\text{-}Ala)_n$ (Traub & Yonath, 1967), $(Gly\text{-}Pro\text{-}Phe)_n$ (Scatturin, Del Pra, Tamburro & Scoffone, 1967) and $(Gly\text{-}Pro\text{-}Lys\text{-}HCl)_n$ which we have recently examined. There is evidence that such polymers are similar to $(Pro\text{-}Gly\text{-}Pro)_n$ in conformation and have the same mode of hydrogen bonding (Traub & Yonath, 1966; Yonath & Traub, 1969).

However, tripeptides with the sequence Gly-Pro-X comprise only 30 to 40% of collagen (Hannig & Nordwig, 1967) and it has been proposed that those regions of the protein where glycine is not followed by proline or hydroxyproline have a different

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conformation in which there are two, rather than one, interchain NH . . . O hydrogen bonds per tripeptide (Ramachandran & Sasisekharan, 1965; Ramachandran, 1967). X-ray studies of $(Gly\text{-}Gly\text{-}Pro)_n$ (Traub, 1969) and $(Gly\text{-}Ala\text{-}Pro)_n$ (Segal & Traub, 1969) have revealed sheet-like structures quite different from that of collagen. However, we have recently obtained collagen-like X-ray patterns from polyhexapeptides incorporating the sequences Gly-Ala-Pro and Gly-Ala-Ala and we have investigated their conformation and mode of hydrogen bonding.

We have studied altogether four polyhexapeptides with the sequences $(Gly\text{-}Pro\text{-}Ala\text{-}Gly\text{-}Pro\text{-}Pro)_n$, $(Gly\text{-}Ala\text{-}Pro\text{-}Gly\text{-}Pro\text{-}Pro)_n$, $(Gly\text{-}Ala\text{-}Pro\text{-}Gly\text{-}Pro\text{-}Ala)_n$ and $(Gly\text{-}Ala\text{-}Ala\text{-}Gly\text{-}Pro\text{-}Pro)_n$. If the two-bonded model for collagen (Ramachandran, 1967) were correct the last three polymers might be expected to form structures with three NH . . . O hydrogen bonds per hexapeptide. $(Gly\text{-}Pro\text{-}Ala\text{-}Gly\text{-}Pro\text{-}Pro)_n$ conforms to the sequence $(Gly\text{-}Pro\text{-}X)_n$ and might be expected to resemble $(Pro\text{-}Gly\text{-}Pro)_n$ in conformation and have only two hydrogen bonds per hexapeptide. However, it has the same composition as $(Gly\text{-}Ala\text{-}Pro\text{-}Gly\text{-}Pro\text{-}Pro)_n$ and we were interested to compare the properties of the two polymers.

The synthesis of the four polyhexapeptides and physico-chemical studies relating to their structure in solution are described in Segal (1969). This paper is devoted to a description of X-ray studies of their conformations in the solid state.

2. Materials and Methods

All polyhexapeptide samples were prepared in the Biophysics Department of the Weizmann Institute. Their synthesis and fractionation are described in Segal (1969). The best specimens for X-ray study were prepared from $(Gly\text{-}Pro\text{-}Ala\text{-}Gly\text{-}Pro\text{-}Pro)_n$ and $(Gly\text{-}Ala\text{-}Pro\text{-}Gly\text{-}Pro\text{-}Pro)_n$ each of 14,000 molecular weight and $(Gly\text{-}Ala\text{-}Pro\text{-}Gly\text{-}Pro\text{-}Ala)_n$ and $(Gly\text{-}Ala\text{-}Ala\text{-}Gly\text{-}Pro\text{-}Pro)_n$ of 6500 and 7600 molecular weight, respectively.

Photographs were taken of powders and oriented films stroked out from aqueous solutions of the polyhexapeptides. Photographs of powders were taken with 114·6 mm diameter cylindrical powder cameras and oriented films were photographed on flat-plate cameras. The flat-plate photographs were standardized by sprinkling a little calcite powder on the specimens or from powder photographs taken of the same specimens.

The spacings of X-ray reflections were measured with a travelling microscope and their intensities with a Joyce–Loebl recording microdensitometer.

3. X-ray Diffraction Patterns

Plate I shows X-ray diffraction patterns obtained from stroked films of the four polyhexapeptides. The specimens all have normal fibre orientation. All the photographs show the main features of the diffraction pattern of collagen, including a meridional 2·9 Å spacing on the tenth layer line and relatively strong reflections on the equator and near the meridian on the third and seventh layer lines.

However, there are variations among the diffraction patterns indicating differences in crystallinity and unit-cell dimensions. $(Gly\text{-}Pro\text{-}Ala\text{-}Gly\text{-}Pro\text{-}Pro)_n$ closely resembles $(Pro\text{-}Gly\text{-}Pro)_n$ (see Yonath & Traub, 1969) in the dimensions and quality of its X-ray pattern. It has the same meridional repeat of 2·87 Å, though the equatorial spacings are slightly shorter than those observed for the polytripeptide. Also, as in the case of $(Pro\text{-}Gly\text{-}Pro)_n$, there is no 110 reflection nor clear evidence of crystalline sampling except on the equatorial and third layer lines. The pattern is thus consistent with the screw disorder and the misalignment of sheets of molecules which we have discussed in Yonath & Traub, 1969. The observed intensities and calculated and observed spacings of $(Gly\text{-}Pro\text{-}Ala\text{-}Gly\text{-}Pro\text{-}Pro)_n$ are shown in Table 1.

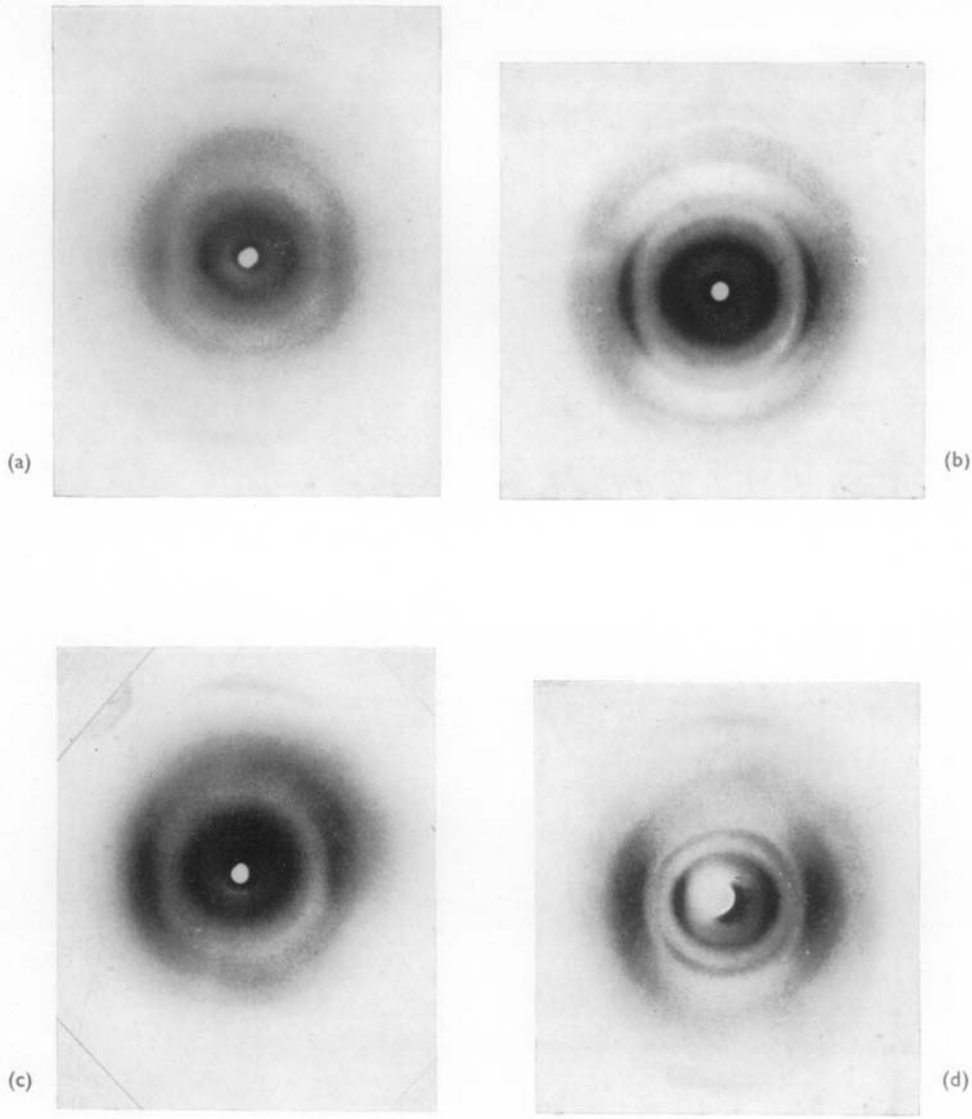

PLATE I. X-ray diffraction patterns of stroked films of polyhexapeptides; (a) (Gly-Pro-Ala-Gly-Pro-Pro)$_n$, (b) (Gly-Ala-Pro-Gly-Pro-Ala)$_n$, (c) (Gly-Ala-Ala-Gly-Pro-Pro)$_n$ and (d) (Gly-Ala-Pro-Gly-Pro-Pro)$_n$. (cf. Plates I and II of Yonath & Traub, 1969.)

[*facing p. 520*

The diffraction patterns of $(Gly\text{-}Ala\text{-}Pro\text{-}Gly\text{-}Pro\text{-}Ala)_n$ and $(Gly\text{-}Ala\text{-}Ala\text{-}Gly\text{-}Pro\text{-}Pro)_n$, which resemble each other closely, appear to be entirely crystalline. As is shown in Tables 2 and 3, we have been able to index all the observed reflections of both patterns in terms of a *c*-axis of 29·5 Å and an *a*-axis of 10·5 Å. This latter dimension apparently corresponds to the average spacing between sheets of molecules. The crystallinity of the pattern and its limitation to reflections of the *h0l* type can be explained by a regular packing of molecules in sheets, which in turn are stacked regularly with respect to their *c* but randomly with respect to their *b*-axes. This situation could occur, for example, if sheets of molecules rolled up to form the type of spiral lattice suggested for collagen by Sasisekharan & Ramachandran (1957).

$(Gly\text{-}Ala\text{-}Pro\text{-}Gly\text{-}Pro\text{-}Pro)_n$ also has a 2·95 Å meridional spacing. We have observed some differences in the X-ray patterns of different preparations of this polyhexapeptide. Though all the patterns are collagen-like, these differences suggest variability in the packing of the molecules. The oriented photograph shown in Plate I(d) has an equatorial reflection corresponding to a 110 spacing, so this form of the polymer probably has hexagonal or pseudo-hexagonal packing. Table 4 shows the intensities and spacings of this diffraction pattern.

In the indexing schemes of Tables 1 to 4, the 2·9 Å meridional reflections are assigned to the tenth layer line and the relatively strong near-meridional reflections appear on the third and seventh layer lines. This implies, in accordance with helical diffraction theory (Cochran, Crick & Vand, 1952), that there are ten equivalent units in three helical turns of the molecular structure. $(Gly\text{-}Pro\text{-}Ala\text{-}Gly\text{-}Pro\text{-}Pro)_n$ thus has an axial translation of 2·87 Å and a rotation of some 108° between equivalent structural subunits, the same helical parameters as were found for $(Pro\text{-}Gly\text{-}Pro)_n$ (see Yonath & Traub, 1969). The helical structures of the other three polyhexapeptides have the same rotation, but a translation of 2·95 Å between equivalent units.

We have measured the intensities of the equatorial reflections of the four polyhexapeptides. It was found for $(Pro\text{-}Gly\text{-}Pro)_n$ (Yonath & Traub, 1969) that these intensities are particularly informative about the mode of interchain hydrogen bonding, and are relatively less affected by water than the intensities on higher layer lines. Intensities were determined from radial microdensitometer traces, as described in Yonath & Traub, 1969. The traces were taken through the 100 and 200 reflections and

TABLE 1

Observed and calculated spacings for $(Gly\text{-}Pro\text{-}Ala\text{-}Gly\text{-}Pro\text{-}Pro)_n$

Orientation	I_o	d_o(Å)	*hkl*	d_c(Å)
Equatorial	vs	10·50	100	10·60
Diagonal	m	7·05	103	7·10
Equatorial	ms	5·40	200	5·30
Diagonal	mw	4·64	203	4·64
Meridional	m	3·94	7th layer	
Meridional	ms	2·87	10th layer	2·87

Observed intensities (I_o) were estimated as very strong (vs), moderately strong (ms), medium (m) and moderately weak (mw). Indices (*hkl*) were assigned to the reflections and their spacings calculated (d_o) on the basis of orthogonal axes with $a = 10{\cdot}6$ Å and $c = 28{\cdot}7$ Å and assuming the 10th but not the 7th layer-line spacing to be meridional.

TABLE 2

Observed and calculated spacings for (Gly-Ala-Pro-Gly-Pro-Ala)$_n$

Orientation	I_o	d_o(Å)	*hkl*	d_c(Å)
Equatorial	vs	10·40	100	10·50
Diagonal	m	7·06	103	7·18
Equatorial	vs	5·31	200	5·25
Diagonal	mw	4·64	203	4·63
Meridional	ms	3·92	107	3·91
Diagonal	w	3·55	206	3·59
Equatorial	vw	3·53	300	3·50
Near meridional	mw	3·33	207	3·29
Meridional	s	2·95	0, 0, 10	2·95
Meridional	w	2·85	1, 0, 10	2·84
Near meridional	vw	2·59	2, 0, 10	2·57

Observed intensities (I_o) were estimated as very strong (vs), strong (s), moderately strong (ms), medium (m), moderately weak (mw), weak (w) and very weak (vw). Indices (*hkl*) were assigned to the reflections and their spacings calculated (d_c) on the basis of orthogonal axes with $a = 10{\cdot}5$ Å and $c = 29{\cdot}5$ Å.

TABLE 3

Observed and calculated spacings for (Gly-Ala-Ala-Gly-Pro-Pro)$_n$

Orientation	I_o	d_o(Å)	*hkl*	d_c(Å)
Equatorial	vs	10·40	100	10·50
Diagonal	mw	7·10	103	7·18
Equatorial	s	5·32	200	5·25
Diagonal	mw	4·56	203	4·63
Meridional	mw	3·95	107	3·91
Equatorial	vw	3·54	300	3·50
Near meridional	w	3·26	207	3·29
Meridional	ms	2·95	0, 0, 10	2·95
Meridional	w	2·84	1, 0, 10	2·84
Near meridional	vw	2·59	2, 0, 10	2·57

Observed intensities (I_o) were estimated as very strong (vs), strong (s), moderately strong (ms), moderately weak (mw), weak (w) and very weak (vw). Indices (*hkl*) were assigned to the reflections and their spacings calculated (d_c) on the basis of orthogonal axes with $a = 10{\cdot}5$ Å and $c = 29{\cdot}5$ Å.

TABLE 4

Observed and calculated spacings for (Gly-Ala-Pro-Gly-Pro-Pro)$_n$

Orientation	I_o	d_o(Å)	*hkl*	d_c(Å)
Equatorial	vs	10·30	100	10·30
Diagonal	m	7·04	103	7·11
Equatorial	ms	5·89	110	5·95
Equatorial	m	5·26	200	5·15
Diagonal	mw	4·51	203	4·56
Meridional	w	4·01	7th layer	
Meridional	m	2·95	10th layer	

Observed intensities (I_o) were estimated as very strong (vs), moderately strong (ms), medium (m), moderately weak (mw) and weak (w). Indices (*hkl*) were assigned to the reflections and their spacings calculated (d_c) on the basis of an hexagonal cell with $a = 11{\cdot}9$ Å and $c = 29{\cdot}5$ Å, and assuming the 10th but not the 7th layer-line to be meridional.

ratios of the integrated intensities for these reflections derived for all the materials except (Gly-Ala-Pro-Gly-Pro-Pro)$_n$. In the latter case traces were taken through 100 and 110, which is better separated from other features of the pattern than is 200, in photographs of this polymer.

For (Gly-Pro-Ala-Gly-Pro-Pro)$_n$ corrections, estimated at some 30%, were made for the contributions of near-meridional streaks to the intensities measured at 200 (see Yonath & Traub, 1969). For the other three polyhexapeptides no attempt was made to correct for streaks, as these do not occur in completely crystalline X-ray patterns, but in the comparison with calculated intensities (see section on Structure Analysis) account was taken of the combined intensity contributions from the equatorial, first and second layer lines. The measured intensity ratios for the four polyhexapeptides are shown in Table 5.

TABLE 5

Observed and calculated equatorial intensity ratios for polyhexapeptides, (Pro-Gly-Pro)$_n$ and collagen

Polymer	Intensity ratio	
	Observed	Calculated
(Gly-Pro-Ala-Gly-Pro-Pro)$_n$	1·6	1·1
(Gly-Ala-Pro-Gly-Pro-Ala)$_n$	2·8	2·1
(Gly-Ala-Ala-Gly-Pro-Pro)$_n$	2·6	2·1
(Gly-Ala-Pro-Gly-Pro-Pro)$_n$	3·3	4·0
(Pro-Gly-Pro)$_n$	0·85	0·88
Collagen	0·60	0·74

Values are given for the 110/100 intensity ratio for (Gly-Ala-Pro-Gly-Pro-Pro)$_n$ and for 200/100 in all other cases. Observed and calculated intensity ratios for (Pro-Gly-Pro)$_n$ and collagen are taken from Yonath & Traub (1969). These and values for (Gly-Pro-Ala-Gly-Pro-Pro)$_n$ refer to equatorial reflections only; values for the other three polyhexapeptides represent summations of unresolved reflections on the equatorial, first and second layer lines.

4. Structure Analysis

The 100 spacings observed for the polyhexapeptides are all between 10 and 11 Å. These dimensions and the close resemblance of their diffraction patterns to those of collagen and (Pro-Gly-Pro)$_n$ strongly indicate that the polyhexapeptides too have three-stranded molecular structures. The drop in molecular weight observed after chemical melting of polyhexapeptides in solution (see Segal, 1969) provides additional support for this conclusion and suggests that each molecule consists of separate polypeptide chains rather than one long chain folded back on itself.

There are two different ways in which three parallel polyhexapeptide chains can aggregate to form structures consistent with the observed helical parameters. The two modes of aggregation are illustrated in Figure 1 for the example of (Gly-Ala-Pro-Gly-Pro-Pro)$_n$. The structural formula of this polymer is shown in Figure 2.

From Figure 1 it can be seen that a translation of 2·9 Å and a rotation of 108° would only relate completely identical units if all tripeptides in all three chains were in fact identical. For the mode of aggregation of Figure 1(a), which is the more regular of the two, the helical parameters are 5·8 Å translation and 216° rotation. For the case of Figure 1(b) a translation of 17·4 Å and a rotation of −72° is required for exact

Chain 1	Chain 2	Chain 3	Chain 1
Gly	Pro	Pro	Gly
Ala	Gly	Pro	Ala
Pro	Pro	Gly	Pro
Gly	Pro	Ala	Gly
Pro	Gly	Pro	Pro
Pro	Ala	Gly	Pro
Gly	Pro	Pro	Gly
Ala	Gly	Pro	Ala
Pro	Pro	Gly	Pro
Gly	Pro	Ala	Gly
Pro	Gly	Pro	Pro
Pro	Ala	Gly	Pro

(a)

Chain 1	Chain 2	Chain 3	Chain 1
Gly	Pro	Pro	Gly
Ala	Gly	Pro	Ala
Pro	Ala	Gly	Pro
Gly	Pro	Ala	Gly
Pro	Gly	Pro	Pro
Pro	Pro	Gly	Pro
Gly	Pro	Pro	Gly
Ala	Gly	Pro	Ala
Pro	Ala	Gly	Pro
Gly	Pro	Ala	Gly
Pro	Gly	Pro	Pro
Pro	Pro	Gly	Pro

(b)

FIG. 1. Two possible modes of aggregation of three polyhexapeptide chains, illustrated for (Gly-Ala-Pro-Gly-Pro-Pro)$_n$, (a) screw axis with translation of 5·8 Å and rotation of 216°, (b) screw axis with translation of 17·4 Å and rotation of −72°.

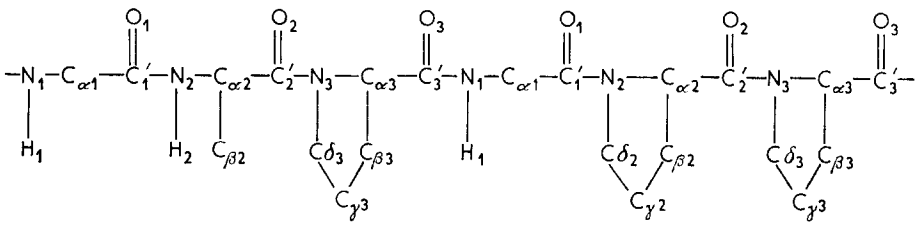

FIG. 2. Structural formula of one hexapeptide unit of (Gly-Ala-Pro-Gly-Pro-Pro)$_n$ indicating notation used in text.

structural correspondence. In the first case, the asymmetric unit of structures is one hexapeptide, and in the second, one hexapeptide from each of the three chains.

Both modes of aggregation could give rise to features in the diffraction pattern, including meridional reflections, in addition to those that would result from a screw axis of 2·9 Å translation and 108° rotation (Cochran *et al.*, 1952). However, as the two halves of the polyhexapeptides differ in only two or four out of eighteen non-hydrogen atoms, such features are likely to be weak and we have not, in fact, observed them.

We have carried out systematic evaluations of possible molecular conformations for the polyhexapeptides in terms of van der Waals contacts and calculated intensities for equatorial reflections, using the procedures described in Yonath & Traub (1969). However, to avoid a prohibitive amount of computation, we have reduced the scope of the analysis on the basis of two simplifying assumptions.

First, we have assumed that the polyhexapeptide structures do not differ very greatly from that found for (Pro-Gly-Pro)$_n$. In terms of the conformational parameters explained in Figure 2 of Yonath & Traub (1969), we have investigated a region of possible structures with $h_1 = 2{\cdot}2$ to $3{\cdot}6$ Å, $h_2 = 2{\cdot}8$ to $3{\cdot}8$ Å, $\mu = 110$ to 150°, $\nu = 80$ to 130°, $\delta_1 = 70$ to 140° and $\rho = 0{\cdot}8$ to $2{\cdot}1$ Å. This region is centred around the structure of (Pro-Gly-Pro)$_n$ and allows a minimum variation in atomic positions of about 1·0 Å for each parameter. The two-bonded and collagen II models are well inside this region, but structures of the collagen I type are excluded.

Second, we have assumed that the α carbon atoms of all the glycine residues in each polyhexapeptide lie on a regular helix, i.e. all these atoms lie at the same radial distance ρ from the helix axis and there are identical translations and rotations relating successive glycyl α carbons. Without this assumption the number of parameters required to describe a polyhexapeptide conformation would be more than double that for the polytripeptide. With this assumption it is possible to analyse polyhexapeptide conformations in terms of two polytripeptides.

Both our postulates are implicit in the generally made assumption that collagen itself incorporates the various amino acid sequences we have considered in an essentially uniform polytripeptide backbone conformation.

We have made systematic conformational analyses, as described in Yonath & Traub (1969), for the four polytripeptide sequences $(\text{Gly-Ala-Pro})_n$, $(\text{Gly-Pro-Ala})_n$, $(\text{Gly-Ala-Ala})_n$ and $(\text{Gly-Pro-Pro})_n$ for triple helices with 2·95 Å axial translation and 108° rotation and for the sequence $(\text{Gly-Pro-Ala})_n$ and $(\text{Gly-Pro-Pro})_n$ assuming 2·87 Å translation and 108° rotation. Substituting alanine for proline residues removes the restrictions on the conformational possibilities imposed by short contacts involving the Cγ and Cδ atoms and by the need of a dihedral angle about the N–Cα bond consistent with closure of the pyrolidine ring. We took account of this even in the analysis of the $(\text{Gly-Pro-Pro})_n$ sequence by ignoring short contacts from the reference tripeptide to Cγ or Cδ atoms of other chains or of second nearest neighbour residues in the same chain, as in the polyhexapeptides these might involve proline–alanine rather than proline–proline interactions. We also allowed 0·5 Å additional latitude in considering short contacts between atoms in neighbouring tripeptides on the same or different chains to allow for the possibility of different conformations in the two halves of a hexapeptide.

Given the assumption we have made, the conformation of a polyhexapeptide can be considered as a combination of possible conformations for the corresponding polytripeptides. Furthermore, structure factors for the polyhexapeptide can be calculated by summing corresponding structure factors for the polytripeptides.

None of the polytripeptide analyses revealed any stereochemically possible conformation with interchain hydrogen bonding of the type $N_1H_1 \ldots O_3$ (see Fig. 2) for which the 200/100 intensity ratio [or 110/100 for $(\text{Gly-Ala-Pro-Gly-Pro-Pro})_n$] is greater than 0·4. As the observed intensity ratios for all the polyhexapeptides are in fact far greater than this (see Table 5), this mode of hydrogen bonding, which is a feature of the two-bonded structure, is clearly excluded by the analyses.

The analyses do reveal stereochemically acceptable conformations with suitably large intensity ratios for all the polytripeptides, but only with hydrogen bonding of the type $N_1H_1 \ldots O_2$, which occurs in $(\text{Pro-Gly-Pro})_n$. Moreover, for all the tripeptide sequences, these acceptable conformations are all quite close to that of $(\text{Pro-Gly-Pro})_n$ and are included in the region with $h_1 = 2{\cdot}6$ to 3·0 Å, $h_2 = 3{\cdot}2$ to 3·6 Å, $\mu = 115$ to 140°, $\nu = 105$ to 125°, $\delta_1 = 80$ to 120° and $\rho = 1{\cdot}4$ to 1·8 Å.

Clearly, by stereochemical criteria, any of the polyhexapeptides could have the same conformation as $(\text{Pro-Gly-Pro})_n$. Indeed, their lower proline content makes the stereochemical restrictions on their possible conformations somewhat less severe.

The $(\text{Pro-Gly-Pro})_n$ conformation is also consistent with the intensities observed for the equatorial reflections of the polyhexapeptides. Table 5 shows comparison of the observed data with intensities calculated for the various polyhexapeptides,

assuming the conformation of $(Pro\text{-}Gly\text{-}Pro)_n$ and water molecules hydrogen-bonded to the free CO groups, as described in Yonath & Traub (1969), as well as to the free NH groups of the alanyl residues. The calculated ratios of equatorial intensities are very sensitive to the spacings of the reflections and also appreciably affected by the contributions of water molecules. They are less affected by contributions of $C\gamma$ and $C\delta$ atoms in the proline ring. As the errors in the intensity measurements may be as much as 30%, and as there may also be considerable errors in the water distribution we have assumed, the agreement between observed and calculated intensity ratios in Table 5 is quite good and supports the conclusion that the various polymers have closely similar conformations. Observed and calculated equatorial intensity ratios for $(Pro\text{-}Gly\text{-}Pro)_n$ and collagen are also shown in Table 5 as well as in Figure 5 of Yonath & Traub (1969), which also illustrates the incompatibility of such high ratios with $N_1H_1 \ldots O_3$ hydrogen bonding.

In fact, at least three of the polyhexapeptides cannot have precisely the same backbone conformations as $(Pro\text{-}Gly\text{-}Pro)_n$, as they have a slightly larger axial translation per tripeptide. The systematic analyses of tripeptide sequences described above have indicated various possible conformations, close to that of $(Pro\text{-}Gly\text{-}Pro)_n$, with an axial translation of 2·95 Å per tripeptide. However, we feel unable to choose among these, as the limited data available to us have not proved adequate to determine the apparently small conformational differences between these polyhexapeptides and $(Pro\text{-}Gly\text{-}Pro)_n$.

Possibly the differences in conformation may result from the fact that these three polyhexapeptides all have an alanine residue following glycine in the amino acid sequence. Whereas for $(Gly\text{-}Pro\text{-}Ala\text{-}Gly\text{-}Pro\text{-}Pro)_n$, with a $(Pro\text{-}Gly\text{-}Pro)_n$-like conformation, the alanine NH would point outwards and could easily form a hydrogen bond with water; NH of alanine in the second position, as in Figure 2 would point towards another polypeptide chain of the same triple helix. Though it is not impossible for a hydrogen bond to be made to water in this position, this might occur more easily if there were a small conformational change.

It is interesting to note that $(Gly\text{-}Ala\text{-}Hypro)_n$ has recently been reported to show a collagen-like X-ray pattern with a meridional spacing of 2·92 Å (Andreeva, Esipova, Millionova, Rogulenkova & Shibnev, 1967), thus supporting a possible correlation between a long meridional spacing and an NH group after the glycyl residue.

5. Discussion

It is clear that all four polyhexapeptides have triple-helical structures with interchain hydrogen bonding of the collagen II type and conformations close to that of $(Pro\text{-}Gly\text{-}Pro)_n$. The physicochemical studies of the polyhexapeptides in solution, described in Segal (1969), provide strong independent evidence for there being only one NH ... O interchain hydrogen bond per tripeptide.

As described in Yonath & Traub (1969), collagen has a relatively high 200/100 intensity ratio, which appears to preclude the $N_1H_1 \ldots O_3$ hydrogen bond of the two-bonded structure, and its intensity distribution is consistent with the $(Pro\text{-}Gly\text{-}Pro)_n$ conformation. As it has now been shown that amino acid sequences of the form Gly-Pro-X, Gly-Ala-Pro and Gly-Ala-Ala can all be incorporated into essentially the same one-bonded conformation, it is difficult to imagine that the helical structure of collagen itself is not also of this type.

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