An analogous situation exists in plants in which large quantities of sucrose are synthesized and secreted by the mesophyll cells. There, sucrose may be synthesized as sucrose phosphate[14,15] and then be hydrolysed to sucrose and inorganic phosphate before it appears in the phloem[15]. The concentration of sucrose phosphate within the cell is never large, whereas in the phloem the concentration of sucrose can approach 20 per cent[16]. A similar mechanism has been proposed for the mammary gland[17], but the intermediate synthesis of lactose phosphate is no longer believed to be the mechanism of lactose synthesis[18,19].

The mechanism(s) by which the mammary gland synthesizes and secretes lactose into the milk therefore remains a problem of general significance. I thank Dr Norman Wessells for his interest and for stimulating discussions. This work was supported by the US Public Health Service and the American Cancer Society.

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On the Molecular Structure of Collagen

by

W. TRAUB
A. YONATH

Department of Chemistry

D. M. SEGAL

Departments of Biophysics and Chemistry,
Weizmann Institute of Science,
Rehovot, Israel

Several polytripeptide and polyhexapeptide models of collagen have the same triple helical conformation with one NH...O interchain H bond per tripeptide. Collagen itself probably has this structure.

We present here a preliminary account of X-ray diffraction and physicochemical studies of several synthetic polytripeptide and polyhexapeptide models for collagen, and discuss the implications of the results for the molecular structure of the protein.

Collagen shows a distinctive X-ray pattern which differs markedly from those of almost all other fibrous proteins. Its principal features include strong first and second orders of an equatorial spacing varying with humidity from 10·5 Å in dry collagen to about 15 Å in wet, a strong meridional arc at 2·9 Å on the tenth layer line, near-meridional reflexions on the third and seventh layer lines and a diffuse distribution of intensity around 4·5 Å chiefly near the equator. In 1955, Ramachandran and Kartha[1] proposed a structural interpretation of this pattern, which has been widely accepted as correct, at least in its general features. The structure is based on a non-integral screw-axis, which relates equivalent units by a translation of 2·9 Å and a rotation of approximately 108°, and there are three helical polypeptide chains, each having every third residue glycine, which are wound around each other to form a three-stranded rope-like structure. Because of the limited detail of the X-ray pattern of collagen and the complexity of its amino-acid sequence, much of which is still unknown, however, the unequivocal elucidation of the precise molecular conformation has proved difficult, and three alternative modifications of the structure have been proposed. They differ in details of the conformation and the mode of interchain hydrogen bonding. In particular, one modification[2] has two systematic hydrogen bonds of the type NH . . . O for every three amino-acid residues, whereas the other two, the so-called collagen I and collagen II structures[3,4,4a], have only one hydrogen bond for three residues.

As an aid to the understanding of the structure and properties of collagen, studies have been made of possible polypeptide models of the protein. In recent years, interest has turned particularly to polytripeptides which have every third residue glycine as well as residues of one or both of the imino-acids proline and hydroxyproline, in accordance with features of the composition of collagen which are believed to play an important part in determining its structure. There is evidence that several such polytripeptides do indeed have collagen-like conformations[5–8], but up to now no detailed structure determination of any of these compounds has been reported. Recently, we have completed a systematic conformational analysis of the polymer $(Gly.Pro.Pro)_n$ (Fig. 1) and arrived at a unique conformation which we believe to be representative of much, if not all, of the helical structure of collagen.

Structure Analysis of $(Gly.Pro.Pro)_n$

$(Gly.Pro.Pro)_n$ gives a well defined X-ray pattern which shows all the principal features of the collagen pattern,

and clearly arises from a three-stranded structure with the same helical parameters as have been found for the protein[6]. In determining its structure, we have had two distinct advantages not available to those who have undertaken a detailed structure analysis of collagen itself. First, we have been able to test various possible conformations in terms of interatomic distances and X-ray intensities calculated for a known chemical formula, rather than some approximation to the overall amino-acid composition of collagen. Second, we have been able to determine the unit-cell dimensions of $(Gly.Pro.Pro)_n$[6]—the cell is hexagonal with $a = 12{\cdot}5$ Å and $c = 28{\cdot}7$ Å—and thus also to test possible conformations by their ability to pack into the available space.

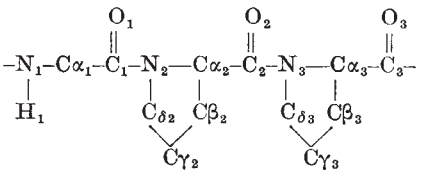

Fig. 1. Structural formula of one tripeptide unit of $(Gly.Pro.Pro)_n$ indicating notation used in text.

The analysis was performed with the aid of the Golem computer of the Weizmann Institute. This was used systematically to generate structures with the observed helical parameters and planar peptide groups with standard bond lengths and angles[9,10] as well as to test these structures in terms of acceptable intramolecular and intermolecular van der Waals contacts and agreement between calculated and observed intensities. It was found convenient to express the polytripeptide conformations in terms of inclinations of lines, joining successive α carbon atoms, to the helix axis and to each other, as well as the orientations of the peptide groups about these lines[11]. These parameters were varied systematically in discrete steps. Van der Waals contacts were tested on the basis of "outer limit" values found in single crystal structure analyses[12]. These limits were reduced by the maximum possible errors in computed interatomic distances, however, due to the finite size of the steps in the parameters and to possible variations either in the conformations of the proline rings or in the nominally tetrahedral angles at the α carbon atoms. An additional requirement imposed was that the glycyl NH group be joined to some carbonyl group by an interchain hydrogen bond of length between 2·6 Å and 3·2 Å and with an HNO angle less than 40°. Such hydrogen bonding is assumed in all three models for collagen and, indeed, it would be hard to imagine a three stranded structure without it.

The analysis was carried out in several stages. In the first, the complete ranges of possible values for the conformational parameters were covered in relatively coarse steps and the conformations tested according to very short van der Waals limits. In subsequent stages, regions found to have structures which passed the tests applied were re-examined at finer intervals and with stiffer criteria. All conformations for which the 2·87 Å translation and 108° rotation between equivalent units follow a right-handed helix were found to have short intramolecular contacts. So were all conformations with a left-handed helix, except for a relatively small region which, however, still includes the parameters of the two-bonded, collagen I, and collagen II models for collagen, in which the NH of glycine makes interchain hydrogen bonds with O_3, O_1 and O_2 of Fig. 1 respectively. The second hydrogen bond of the two-bonded structure is of the type $N_2H_2 \ldots O_2$ and implies an amino rather than an imino-acid residue after glycine. All conformations with hydrogen bonding of the collagen I type that were not excluded by intramolecular criteria turned out to have short intermolecular contacts. On the other hand, all conformations with N_1H_1 hydrogen bonded to O_3 were found to give ratios of the calculated intensities for the 200 and 100 reflexions which are less than half the observed value of 0·85. Both 100 and 200 are strong low-order reflexions which are sensitive only to fairly gross changes of structure, and so the ratio of their intensities is a reliable structural criterion. Furthermore, conformations with this type of hydrogen bond were all found to have HNO angles near 40° and C_2O_2 carbonyl groups pointing inwards towards the helix axis and unavailable for hydrogen bonding even to water.

The only type of conformation found consistent with all the data has $N_1H_1 \ldots O_2$ hydrogen bonding, with the NH pointing clockwise when viewed from the carboxyl ends of the chains. Table 1 shows atomic coordinates for what seems to be the best structure by all criteria. The bond lengths and angles are in agreement with accepted values and all intramolecular and intermolecular van der Waals distances are greater than the normal limits[12]. Fig. 2 shows a comparison of observed intensities with those calculated for this structure, with and without the addition of two water molecules per tripeptide in positions where they form hydrogen bonds to the two carbonyl groups not connected with NH. The structure we have derived for $(Gly.Pro.Pro)_n$ resembles collagen II in its mode of hydrogen bonding, but there are appreciable differences between our atomic coordinates and those previously reported. Consequently there are differences in the calculated intensities and in the length of the NH ... O hydrogen bond. Our value of 2·96 Å is considerably longer than the 2·85 Å and 2·70 Å previously reported[3,14], but conforms better with commonly observed NH ... O hydrogen bonds and the indication, from infrared data, of a long hydrogen bond in collagen[15].

Table 1. ATOMIC COORDINATES OF $(GLY.PRO.PRO)_n$

Atom	x (Å)	y (Å)	z (Å)	r (Å)	φ (deg)
N_1	1·65	−1·11	−0·96	1·99	−33·8
H_1	0·86	−1·70	−1·08	1·91	−63·1
$C\alpha_1$	1·46	0·00	0·00	1·46	0·0
C'_1	2·59	0·16	1·03	2·59	3·5
O_1	3·23	−0·80	1·44	3·33	−14·0
N_2	2·76	1·38	1·56	3·09	26·6
$C\alpha_2$	3·79	1·64	2·60	4·13	23·4
$C\beta_2$	3·92	3·15	2·63	5·03	38·8
$C\gamma_2$	2·63	3·66	2·01	4·51	54·4
$C\delta_2$	2·01	2·57	1·14	3·26	51·9
C'_2	3·31	1·08	3·94	3·48	18·2
O_2	2·21	1·47	4·36	2·66	33·6
N_3	4·03	0·23	4·66	4·04	3·3
$C\alpha_3$	3·58	−0·35	5·94	3·60	−5·6
$C\beta_3$	4·83	−1·08	6·37	4·95	−12·6
$C\gamma_3$	5·72	−1·37	5·17	5·88	−13·5
$C\delta_3$	5·35	−0·28	4·16	5·36	−3·0
C'_3	2·97	0·63	6·92	3·04	12·0
O_3	3·37	1·80	7·02	3 82	28·2
$C\alpha_4$	1·18	0·86	8·61	1·46	36·0
$(H_2O)_1$	3·58	−1·46	9·09	3·87	−22·2
$(H_2O)_2$	2·86	−3·92	7·98	4·85	−53·9

The dihedral angles[13] are $\varphi = 128{\cdot}5°$, 104·2° and 134·6° and $\psi = 333{\cdot}4°$, 306·8° and 328·1° at $C\alpha_1$, $C\alpha_2$ and $C\alpha_3$ respectively.

Structures of Other Polytripeptides

Several other polytripeptides of the form $(Gly.Pro.X)_n$, including $(Gly.Pro.Hypro)_n$[5], $(Gly.Pro.Ala)_n$[7], $(Gly.Pro.Phe)_n$[8] and $(Gly.Pro.Lys.HCl)_n$, show collagen-like X-ray patterns and their spacings indicate that they may have the same conformation as $(Gly.Pro.Pro)_n$[6]. In any case, because these polymers have proline after glycine, they could not form the two-bonded structure and even a single $N_1H_1 \ldots O_3$ hydrogen bond would imply the very unlikely HNO angle and C_2O_2 orientation described. Tripeptides with the sequence Gly.Pro.X comprise some 30 to 40 per cent of collagen[16] and seem to be concentrated largely on adjacent portions of all three chains[6]. Such regions of the protein would therefore have a one-bonded

structure and probably the same backbone conformation as (Gly.Pro.Pro)$_n$.

We have investigated two polytripeptides, (Gly.Gly.Pro)$_n$ and (Gly.Ala.Pro)$_n$, which have an amino rather than an imino-acid residue after glycine, but have not found a collagen-like triple-helical structure in either case. Both these polymers form structures in which chains, with conformations of the polyproline II type[17], rather than being wound around each other, are hydrogen bonded together to form extended sheet-like aggregates. In (Gly.Gly.Pro)$_n$ the chains are held together by two NH . . . O hydrogen bonds per tripeptide to form double-layered sheets[18]. In (Gly.Ala.Pro)$_n$ there is only one NH . . . O hydrogen bond per tripeptide and the chains form a corrugated sheet.

Structure Analysis of Polyhexapeptides

We have, however, obtained collagen-like X-ray patterns from four polyhexapeptides with the sequences (Gly.Pro.Ala.Gly.Pro.Pro)$_n$, (Gly.Ala.Pro.Gly.Pro.Pro)$_n$, (Gly.Ala.Ala.Gly.Pro.Pro)$_n$ and (Gly.Ala.Pro.Gly.Pro.Ala)$_n$. All show the principal features of the diffraction pattern of the protein, including a meridional 2·9 Å spacing on the tenth layer line, and relatively strong reflexions on the equator and near the meridian on the third and seventh layer lines. If the two-bonded structure for collagen were correct, the last three polymers might be expected to form structures with three NH . . . O hydrogen bonds per hexapeptide. (Gly.Pro.Ala.Gly.Pro.Pro)$_n$ conforms to the sequence (Gly.Pro.X)$_n$ and might be expected to resemble (Gly.Pro.Pro)$_n$ in conformation. 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 reflexions, using the procedures that were applied to (Gly.Pro.Pro.)$_n$. We have only considered conformations with $N_1H_1 \ldots O_2$ or $N_1H_1 \ldots O_3$ interchain hydrogen bonding, on the assumption that the polyhexapeptide structures do not differ very greatly from that found for (Gly.Pro.Pro)$_n$. We have also assumed that the α carbon atoms of all the glycine residues in each polyhexapeptide lie on a regular helix. 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. The 200/100 intensity ratios observed for the polyhexapeptides are considerably larger even than for (Gly.Pro.Pro)$_n$, but the analyses revealed no stereochemically possible conformation with $N_1H_1 \ldots O_3$ bonding for which the 200/100 ratio [or 110/100 for (Gly.Ala.Pro.Gly.Pro.Pro)$_n$] is greater than 0·4. Consequently, this mode of hydrogen bonding, which is a feature of the two-bonded structure, is clearly excluded. On the other hand, the analysis of the structures with $N_1H_1 \ldots O_2$ hydrogen bonding did reveal stereochemically acceptable conformations with suitably large intensity ratios for all the polyhexapeptides (see Table 2). All these acceptable conformations are quite close to that of (Gly.Pro.Pro)$_n$.

Physicochemical Studies

For most of the polymers we have investigated, the results of the X-ray studies have been supported by physicochemical studies concerning the structures of the polymers in solution. Neither (Gly.Ala.Pro)$_n$ nor (Gly.Gly.Pro)$_n$ is readily soluble in water, but the latter has been reported to have a highly associated ordered structure in 1·4 M acetic acid[19]. (Gly.Pro.Pro)$_n$[20] and the four polyhexapeptides show collagen-like sigmoidal temperature transitions in aqueous solution. The transitions are dependent on molecular weight and can also be effected by addition to the solutions of guanidinium chloride. The molecular weights of (Gly.Ala.Pro.Gly.Pro.Pro)$_n$ and (Gly.Pro.Ala.Gly.Pro.Pro)$_n$ in 5 M guanidinium chloride were found to be about a quarter of the values observed in 0·2 M NaCl, indicating that the ordered polyhexapeptide structures are, in fact, multi-stranded. The transition temperature should be related to the amount of interchain hydrogen bonding. If some of the polymers do have more than one hydrogen bond per tripeptide this should provide additional stabilization of their structures and one would expect, for example, that (Gly.Ala.Pro.Gly.Pro.Pro)$_n$ would have a much higher transition temperature than (Gly.Pro.Ala.Gly.Pro.Pro)$_n$[21]. In fact, the four polyhexapeptides did not show large differences in transition temperature, and for fractions of equal molecular weight (11,500) a somewhat lower value was found for (Gly.Ala.Pro.Gly.Pro.Pro)$_n$ (32° C) than for (Gly.Pro.Ala.Gly.Pro.Pro)$_n$ (49° C). These results are quite consistent with a one-bonded structure for all the polymers.

More direct evidence regarding the amount of hydrogen

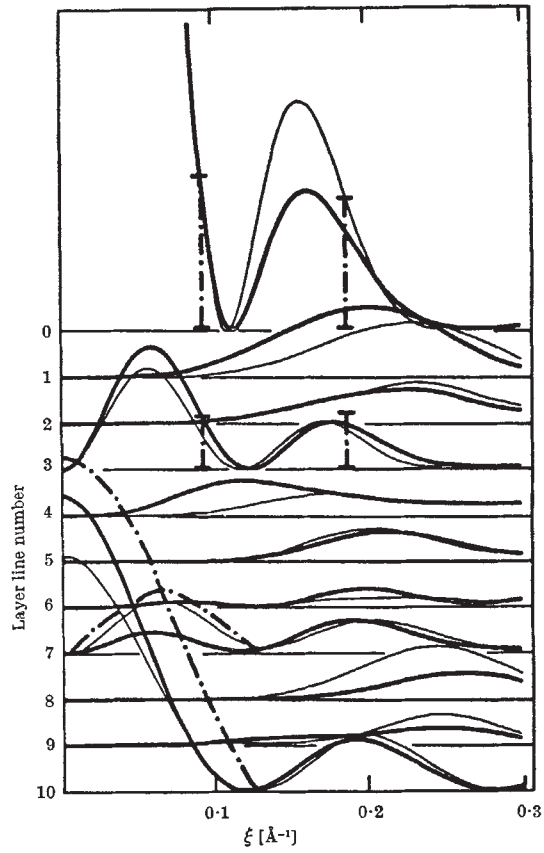

Fig. 2. Comparison of observed and calculated intensities for (Gly.Pro.Pro)$_n$. The observed intensities, shown by dashed lines, were measured with a recording microdensitometer. The specimens showed evidence of screw disorder, so a distinction was made between sampled crystalline reflexions on the equatorial and third layer lines and streaks on other layer lines[23]. Light and heavy continuous lines show intensities calculated, for a cylindrically averaged Fourier transform[24], with and without water contributions respectively. We have not measured the near-equatorial diffuse intensity around 4·5 Å. This would correspond chiefly to the broad calculated peaks near this spacing on the first and second layer lines. The calculated curves have been scaled in accordance with the observed intensity of 100.

Table 2. OBSERVED AND CALCULATED EQUATORIAL INTENSITY RATIOS AND OBSERVED 100 SPACINGS (d_{100}) FOR POLYHEXAPEPTIDES (GLY.PRO.PRO)$_n$, AND COLLAGEN

Polymer	Intensity ratio Observed	Calculated	d_{100} (Å)
(Gly.Pro.Ala.Gly.Pro.Pro)$_n$	1·6	1·1	10·5
(Gly.Ala.Pro.Gly.Pro.Ala)$_n$	2·8	2·1	10·4
(Gly.Ala.Ala.Gly.Pro.Pro)$_n$	2·6	2·1	10·4
(Gly.Ala.Pro.Gly.Pro.Pro)$_n$	3·3	4·0	10·3
(Gly.Pro.Pro)$_n$	0·85	0·88	10·85
Collagen	0·60	0·74	11·5

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. All intensities were calculated from the atomic coordinates of Table 1, with appropriate selections for the various amino-acid sequences of the polyhexapeptides.

bonding in the polyhexapeptides has been provided by tritium-hydrogen exchange studies. These were performed by the two-column method of Englander[22] at 0° C. $(Gly.Ala.Pro.Gly.Pro.Pro)_n$ of molecular weight 28,000, $(Gly.Pro.Ala.Gly.Pro.Pro)_n$ of molecular weight 23,000 and $(Gly.Ala.Pro.Gly.Pro.Ala)_n$ of molecular weight 5,400 were studied at *p*H 3·5 and the two former polyhexapeptides also at *p*H 4·5. At *p*H 4·5 both exchange curves have an initial non-linear part and then a linear portion with a very low slope. When the linear portions were extrapolated to zero time, it was found that $(Gly.Ala.Pro.Gly.Pro.Pro)_n$ and $(Gly.Pro.Ala.Gly.Pro.Pro)_n$ have respectively 2·0 and 2·1 very slowly exchanging hydrogens per hexapeptide. At *p*H 3·5 even freely exposed peptide hydrogens exchange fairly slowly and all the peptide hydrogens in the three polymers could be accounted for. Kinetic analysis of the exchange curves as a sum of independent first-order reactions[22] indicated, however, that in every case two peptide hydrogens per hexapeptide exchange appreciably more slowly than the others. It seems clear that these two are involved in NH . . . O interchain hydrogen bonding.

Conclusions

Our studies show that a three stranded structure with the helical parameters that have been found for collagen can be formed with only one interchain hydrogen bond per tripeptide of the form $N_1H_1 \ldots O_2$. Furthermore, amino-acid sequences of the form Gly.Pro.Pro, Gly.Pro.Ala, Gly.Ala.Pro and Gly.Ala.Ala can all be incorporated into essentially the same conformation with this mode of hydrogen bonding. Even $(Gly.Ala.Pro)_n$, which has a sequence appropriate to the standard two-bonded model[2,15], forms a stable one-bonded structure, though not of the collagen type. It is difficult to imagine amino-acid sequences in collagen which might differ in interchain hydrogen bonding propensities from all the polymers we have studied.

The intensity distribution of the collagen X-ray pattern[3] is similar to that of $(Gly.Pro.Pro)_n$ and can be quite well accounted for by the calculated intensities shown in Fig. 2. Furthermore, the relatively high 200/100 intensity ratio of 0·6, which we have observed in collagen with a 100 spacing of 11·5 Å, appears to be incompatible with the two-bonded model[15]. It therefore seems probable that the conformation of $(Gly.Pro.Pro)_n$ is, in fact, representative of the helical structure of collagen as a whole.

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Further Microwave Emission Lines and Clouds of Ammonia in our Galaxy

by
A. C. CHEUNG
D. M. RANK
C. H. TOWNES
Department of Physics
W. J. WELCH
Radio Astronomy Laboratory and
Department of Electrical Engineering,
University of California, Berkeley

Further NH_3 emission has been found in the Sagittarius region, giving clues to the conditions in dust clouds.

DETECTION of microwave emission from NH_3 in the direction of Sgr *A* has already been reported[1]. The ratio of the weakly detected (2,2) line to the stronger (1,1) line gave a temperature for the NH_3 cloud of about 23° K, and the intensity of (1,1) radiation gave an NH_3 column density of about 2×10^{16} cm^{-2} and a cloud size of about 3′. We report here additional clouds in the Sgr region in which NH_3 has been found, and detection of emission from several more lines of the NH_3 inversion spectrum in one of these. The work was carried out, as before, with the 20 foot millimetre-wave radio telescope at the Hat Creek Observatory described by Cheung *et al.*[1].