Hypothesis

A NOVEL STRUCTURAL MODEL FOR COLLAGEN: WATER–CARBONYL HELIX

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1. Introduction

During the past thirty years several models of the spatial structure for collagen and related synthetic polypeptides have been proposed (see, e.g. [1–16]). In these models the three polypeptide chains of collagen are joined by interchain hydrogen bonds and van der Waals' contacts. The opposite principle was used for the building of our structure of collagen. In our structure there are no interchain hydrogen bonds and van der Waals' contacts and a triple-helical structure is stabilized by water molecules which form interchain bridges through hydrogen bonds with carbonyl groups.

2. Water–carbonyl helix

The collagen triple helix is made up of three chains with the 'collagenous' sequence $(\text{Gly–X–Y})_n$ where X and Y stand for any amino acid residue including proline and hydroxyproline [17]. In our structure, each chain forms a regular helix (see fig.1) which will be referred to as a major helix. This major helix can be transformed into the known major helix in collagen II [6,12,15] by rotating all the $C^{\alpha}_{Y}C^{\alpha}_{Gly}$ peptide groups (peptide groups situated between the C^{α}-atoms of Y and glycine residues) through 180° about the $C^{\alpha}_{Y}–C^{\alpha}_{Gly}$ lines. Three major helices are joined by a left-handed water–carbonyl helix which is formed by water molecules and the X- and Y-carbonyl oxygens (see fig.2). Adjacent turns of the water–carbonyl helix (see fig.2) are bound by water bridges. Water molecules hydrogen-bonded with carbonyl oxygens will be described here as helical water molecules, those water molecules binding the adjacent turns of the helix as interturn water molecules, and a linear chain of helical and interturn water molecules as a water ridge. The helix in fig.2 is energetically the most advantageous among other sterically allowed water–carbonyl helices. In this helix, the oxygen atom of each helical water molecule is at the centre of a tetrahedron formed by two carbonyl oxygen atoms and two oxygen atoms of

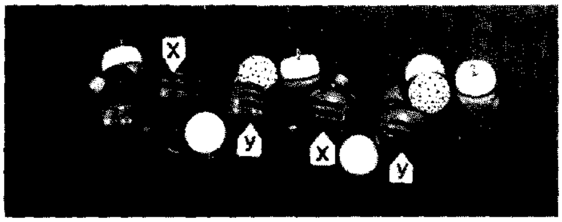

Fig.1. Major helix: An asymmetrical unit of the helix is a triplet (Gly–X–Y) with the angles: $\varphi_{Gly} \simeq 100°$, $\Psi_{Gly} \simeq 180°$; $\varphi_X \simeq -60°$, $\Psi_X \simeq 150°$; $\varphi_Y \simeq -60°$, $\Psi_Y \simeq -20°$. H^{β}-atoms and carbonyl oxygens of glycine residues are dotted. Symbols X and Y mark carbonyl oxygens in the X- and Y-positions of triplets. X- and Y-carbonyl oxygens are located at an equal distance from each other and form the helix with a slight right-handed twist.

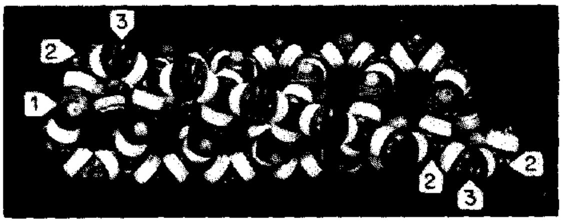

Fig.2. Water–carbonyl helix: 1 and 2 are carbonyl oxygen and helical water molecule, respectively. The adjacent turns of the helix are bound by interturn water molecules (3) which form hydrogen bonds with helical water molecules (2). Helical and interturn water molecules form three water ridges. One of them (2,3 . . . 2,3,2) is well defined in the foreground.

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interturn water molecules. Such an arrangement of helical water molecules allows each of them to form four optimal hydrogen bonds [18]. Construction of water–carbonyl helices with two, four and more major helices is sterically impossible.

3. Water–carbonyl structure for collagen

With the water–carbonyl helix the major helices can form two types of triple helices – symmetrical and asymmetrical (see fig.3). Both in the symmetrical and the asymmetrical triple helices the water ridges are situated between major helices and the length of a triplet (Gly–X–Y) is equal to the length of a water ridge fragment consisting of three helical and two interturn water molecules. The carbonyl oxygen of each glycine residue is able to form a water bridge (a glycine water molecule) with every second interturn water molecule of the water ridge (see fig.3(a,b)). Major helices in both triple helices are not in contact with each other and interact in the same way with water ridges, as the shift of a major helix by one or several turns along the axis of the water–carbonyl helix does not result in a change of its arrangement relative to the water ridges (see fig.2,3). Therefore the symmetrical and asymmetrical triple helices are energetically equivalent. Evidently, the choice of one of them can be done in the course of collagen assembly by a specific interaction of terminal chain regions

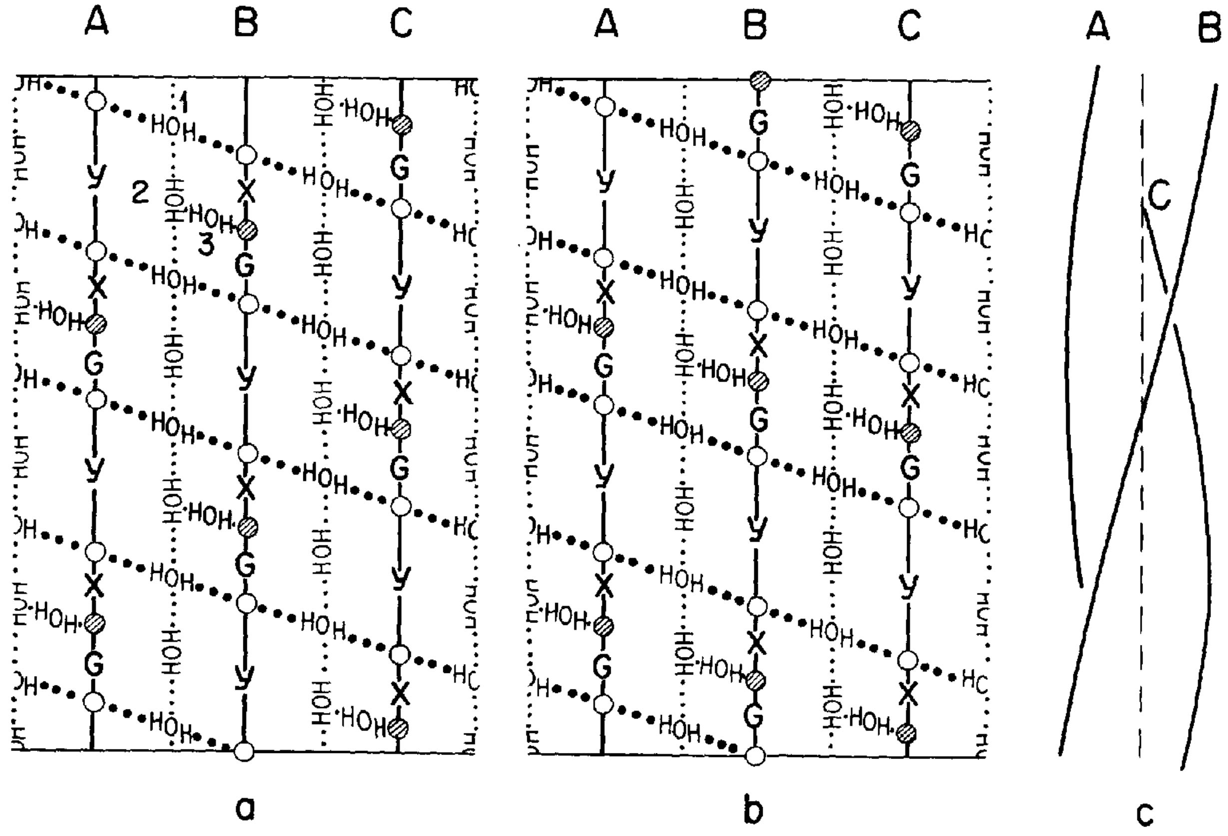

Fig.3. Diagram showing formation of the collagen triple helix from three major helices A, B and C. (a) Symmetrical triple helix: Helix B and helix C are shifted upwards relative to helix A by, respectively, 1/3rd and 2/3rds of the height of a triplet (GLy–X–Y). Open circles denote carbonyl oxygens in the X- and Y-positions of a triplet. Shaded circles denote carbonyl oxygens of glycine residues. 1, 2 and 3 are helical, interturn and glycine water molecules, respectively. Dots represent hydrogen bonds. Large dots represent hydrogen bonds forming the water–carbonyl helix. Major helices A, B and C are related to one another by a 3-fold screw axis with a right-handed twist. (b) Asymmetrical triple helix: Helix B and helix C are shifted downwards relative to helix A by, respectively, 1/6th and 2/6ths of the triplet height. This triple helix does not have a rotational symmetry. (c) Arrangement of major helices A, B and C in triple helices: Dashed line represents the axis of a triple helix having a right-handed twist.

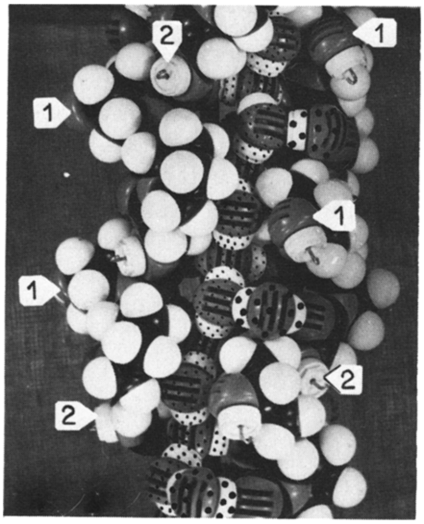

Fig.4. Symmetrical water–carbonyl structure assembled from the polymer (Gly–Pro–Hyp)$_n$. Two adjacent chains of the triple helix face the reader. Small dots show water ridge molecules and large dots, glycine water molecules. (1) OH-group of hydroxyproline; (2) NH-group of glycine residue.

which are not included in the triple helix [19]. In the following we will consider only the symmetrical triple helix, since in stereochemical properties these structures are also practically equivalent. We will call this type of structure a water–carbonyl structure of collagen.

Figure 4 presents the symmetrical water–carbonyl structure with the amino acid sequence (Gly–Pro–Hyp)$_n$ assembled from CPK atomic models. Table 1 lists structural parameters for this model and its water–carbonyl helix. In this model the three major helices themselves are related by a right-handed screw symmetry, which may be designated by a rotation of 131.8° about the axis of the triple helix, followed by a translation of 2.85 Å along the same axis. The water–carbonyl structure has the form of a cylinder with a hole along the axis (d = 5.1 Å). Van der Waals' diameter of this hole is ~2 Å. The hole is formed by the X- and Y-carbonyl oxygens located at a distance of 2.55 Å from the triple helix axis (see table 1). The distance between adjacent major helices is about 10–12 Å. The H^β-atom of each glycine residue is directed towards the centre of the triple helix. Moreover the H^β-site is very close to the water ridge and is situated in such a way that no residue, except glycine, can be accommodated even if the triple helix is deformed.

All X- and Y-side chains are located on the outer surface of the triple helix. We have analyzed the stereochemical behaviour of side chains in the X- and Y-positions of triplets in the presence of water ridges and glycine water molecules. This analysis has shown that side chains of all residues can be located sterically in the X- and Y-positions of triplets. Hydrophilic side chains can form hydrogen bonds and water bridges with glycine and interturn water molecules. For instance, when hydroxyproline is located in a Y-position, its OH-group can form a hydrogen bond with

Table 1
Helical parameters of a collagen model

Helices	Units[a] per turn	Unit[a] twist (deg.)	Unit[a] height (Å)		
				r_1 (Å)	2.55
				r_2 (Å)	2.98
Water–carbonyl	1.58	228.20	2.85	r_3 (Å)	4.63
				χ_1 (deg.)	104.78
Major	10.17	35.43	8.55		
				χ_2 (deg.)	140.75

[a] A unit of the water–carbonyl helix is one X-carbonyl oxygen, one Y-carbonyl oxygen, two helical and two interturn water molecules (see fig.3(a)). A unit of a major helix is a triplet (Gly–X–Y)

r_1, r_2 and r_3 are distances from the axis of the triple helix to X- and Y-carbonyl oxygens, the oxygen of a helical water molecule and the oxygen of an interturn water molecule, respectively. The length of all hydrogen bonds is 2.76 Å

χ_1 is an angle O_h–O_I–O_h where O_h and O_I are oxygens of helical and interturn water molecules, respectively

χ_2 is an angle O_h–O_c–O_h where O_c is X- and Y-carbonyl oxygens

Angles O_c–O_h–O_c, O_I–O_h–O_I ad O_I–O_h–O_c are equal to 109.5°

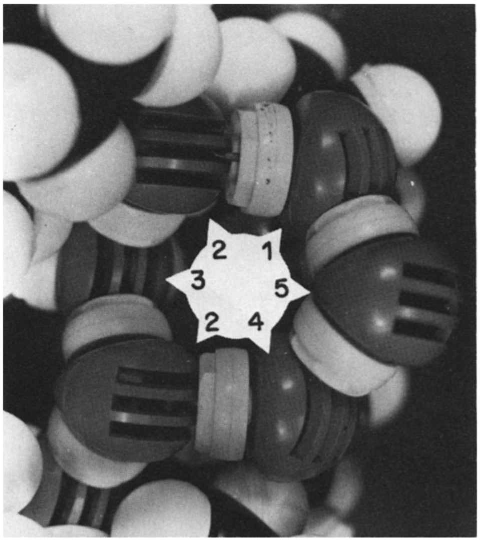

Fig.5. Interaction of the hydroxyproline OH-group with interturn and glycine water molecules: (1) the hydroxyproline OH-group; (2,3,4) interturn, helical and glycine water molecules, respectively; (5) a water bridge between 1 and 4.

the interturn water molecule and a water bridge with the glycine water molecule. As a result, a ring-structure can be formed as is observed in ice (see fig.5).

This analysis has also revealed that within regions (X–Y–Gly) bulky side chains located in X- and Y-positions, as a rule, set up steric difficulties for a good hydrogen bonding between a glycine NH-group and water molecules (see fig.4).

Interaction of interturn and glycine water molecules with other water molecules will lead to an appearance of an ice-like layer on the surface of the water–carbonyl structure. The number of ordered water molecules will be at least five within a triplet. Five is the minimal number of water molecules (two helical, two interturn and one glycine water molecules) which hold together the major helices within the length of one triplet (see fig.3(a,b)). It is interesting to note that the presence of all hydrophobic side chains on the surface of the water–carbonyl structure of collagen will not result in a significant loss of hydrophobic interactions, as hydrophobic side chains are surrounded by water molecules ordered by other elements of the triple helix.

The water–carbonyl structure can change its parameters by distortion of water ridges. For instance, to double the right-handed twist of the water–carbonyl structure, it is sufficient to disturb slightly (by about 20°) the tetrahedral arrangement of the hydrogen bonds.

As noted above, our major helix can be transformed into the major helix of collagen II by the rotation of the intertriplet peptide groups. At the same time, it is shown in [13] that the rotation of the intertriplet peptide groups is sterically possible. This feature of the water–carbonyl structure is of great interest, since it permits the assumption that in solution under certain conditions the transition water–carbonyl structure $\leftrightarrows$ collagen II can take place.

4. Discussion

The spacings of our triple helix of collagen (see fig.3,4 and table 1) and the spacings which may be obtained by a slight distortion of the water–carbonyl helix are compatible with the X-ray data [6,14,15]. For instance, in the two-dimensional electron density maps for the crystalline polymer $(\text{Gly–Pro–Pro})_{10}$ [14], the center portion of the triple helix has low electron density, and high electron density parts are distributed cylindrically with a diameter of about 5.7 Å. These data are completely compatible with a very high electron density of oxygen atoms of the water–carbonyl helix (cp. the diameter of the water–carbonyl helix in table 1). The maps also show that an increase of the diameter of the hole along the axis of the triple helix is accompanied by a decrease of the X-ray R-index. Therefore, an increase in diameter of the hole to the value 5.1 Å in our model should result in a significant decrease of the R-index. In [14] only the structures with diameter of the hole not exceeding 3 Å were investigated, as a further increase of the diameter leads to a disruption of interchain peptide hydrogen bonds, and such structures were not considered in the paper.

The data in [20] showed the existence of ordered water at a distance of 3 Å from the collagen axis. In our structure the helical water molecules are situated at such a distance from the axis (see table 1). The data on IR dichroism, NMR and thermodynamic measurements are also compatible with the water–carbonyl structure of collagen (reviews [8,17,21–23]).

Angles φ and Ψ of our major helix (see fig.1) were derived from the atomic model of collagen (see fig.4). Therefore, their values are given with an accuracy of only ±20°. Within the limits of such an error the conformation of our major helix is very similar to conformation N38 in table 4 of [16], which has $\varphi_{Gly} = 85^\circ$, $\Psi_{Gly} = -172^\circ$; $\varphi_X = -75^\circ$, $\Psi_X = 164^\circ$; $\varphi_Y = -75^\circ$, $\Psi_Y = -46^\circ$. This conformation is one of the few energetically acceptable conformations and which also satisfies X-ray data.

Of particular interest is the localization of the amide A absorption band [21,22,24] in IR spectra of dry and wet collagens and synthetic polypeptides related to collagen. The amide A frequency for these structures is about 40–60 cm^{-1} higher than that for α-helices, β-structures and random coils. It is suggested that a high-frequency shift might be due to the existence of weak hydrogen bonds [21,22,24]. As mentioned above, such hydrogen bonds in the water–carbonyl structure are the hydrogen bonds formed by glycine NH-groups and water molecules. When water is removed from the water–carbonyl structure by drying, glycine NH-groups can form only deformed interchain hydrogen bonds. These groups can form good hydrogen bonds only in a structure of the collagen II type. The water–carbonyl structure, on drying, cannot adopt a structure of the collagen II type. The transition water–carbonyl structure → collagen II must be accompanied by a displacement of the major helices relative to each other along the axis of the triple helix. Such a displacement is possible in solution but is impossible during drying of the water–carbonyl structure. Thus, we see that at any humidity in our structure there are weak hydrogen bonds which are responsible for the high-frequency shift of the amide A.

As noted in the previous section the water–carbonyl structure can be transformed into collagen II. Therefore it is possible that, in the native structure of collagen, along with water–carbonyl structure there exist fragments of the collagen II type of structure (this idea was proposed to the author by Drs N. G. Esipova and V. G. Tumanyan).

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