HYDROXYPROLINE STABILIZES BOTH INTRAFIBRILLAR STRUCTURE AS WELL AS INTER-PROTOFIBRILLAR LINKAGES IN COLLAGEN*

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ABSTRACT

It is shown that the previous report from the author's laboratory that hydroxyproline has a role in stabilizing collagen structure can be slightly modified to make it serve the purpose of not only leading to hydrogen bonds between two chains in the triple helix, but also in linking one triple helix with another. Tht modified scheme of hydrogen bonding is discussed and illustrated with diagrams.

IN a recent paper[1], it was reported from our laboratory that hydroxyproline can play an important part in stabilizing the collagen structure by forming hydrogen bonds, with its hydroxyl group ($O^{\gamma}H^{\gamma}$) playing a vital role in this linkage. The essence of the type of hydrogen-bonded linkages that was proposed is continued in the diamond shaped region shown in Fig. 1 of that paper. A more careful re-examination of this structure indicates that it would be possible for the hydroxyproline OH to be involved both in a hydrogen bond connecting two neighbouring chains (say A and B) in the triple helix, as well as to form a hydrogen bond with a neighbouring triple-helical protofibril. This arrangement of hydrogen bonds is shown in Fig. 1, where only the relevant atoms of chain A and B are shown in detail. It will be seen that the NH group (N_2H_2), corresponding to the second residue in the sequence –Gly–X–Y– which occurs in collagen, forms a hydrogen bond with O_1 of a neighbouring chain *via* the water molecule O^w (H_1^w, H_2^w). Of the two protons in the water, one forms an almost straight hydrogen bond with O_1 of chain A, while the second proton forms a hydrogen bond with the O_3^{γ} atom of chain A acting as a receptor for this bond (the standard nomenclature of referring to all atoms belonging to the same residue by the same subscript is adopted in this paper, which is slightly different from the earlier notation of denoting all atoms in the same peptide unit by the same subscript, which was adopted in Ref. 1). It will be seen, therefore, that the water molecule does not have any free proton available for external linkage in this model. For the same reason, the water medium in the structure cannot easily disturb the water proton by forming a hydrogen bond *via* that atom.

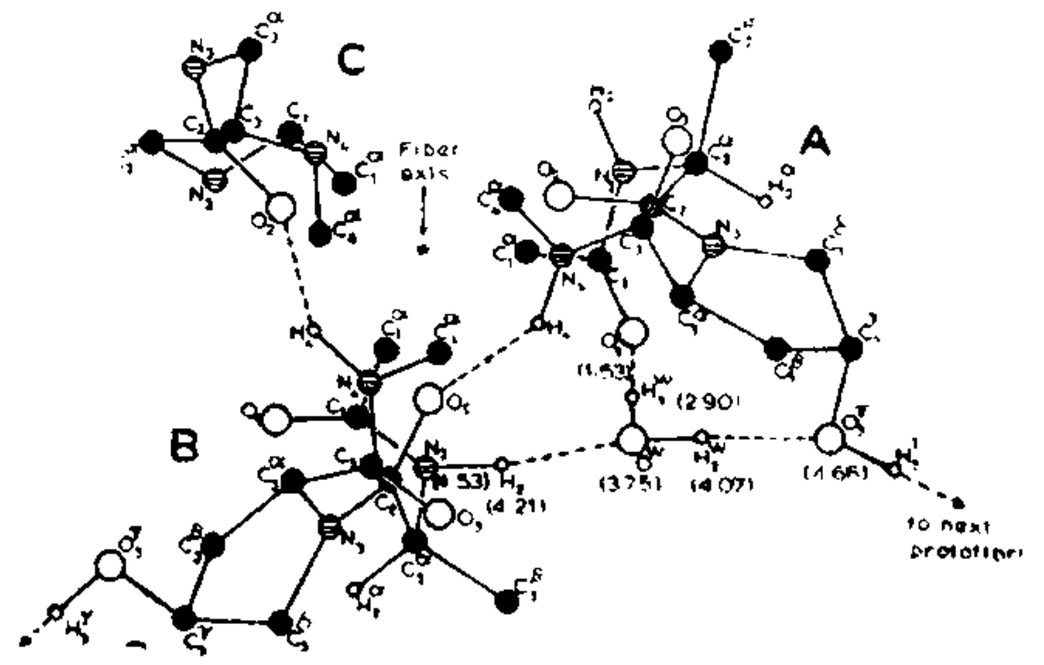

FIG. 1. Diagram showing the hydrogen bonding arrangement in a protofibril of collagen involving the hydroxyproline OH group and a water molecule. The water molecule is linked differently with the Hyp O^{γ} from what was previously reported in Ref. 1. Here the water donates a proton to O^{γ} and the O^{γ} proton is the donor of the hydrogen bond linking the protofibril chain to a neighbouring protofibril. (The numbers denote the heights of the atoms concerned parallel to the fibre axis.)

What is more interesting is the fact that the proton H_3^{γ} of the hydroxyl group in residue 3 of chain A is now available for linking one protofibril with another. This is shown in Fig. 2 in which the linking hydrogen bond from Hyp for a central protofibril is shown with three other protofibrils in outline in the neighbourhood of this. The diagram shown corresponds to the case in which the hydrogen bond is direct between O_3^{γ} H_3^{γ} of the central protofibril and the O_3 of the nieghbouring protofibril. The distance between the two proto-

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fibrils corresponds to 12·5 A, which comes very close to the lattice constant to dry collagen. It is interesting to note that the orientation of the proton of the γ-hydroxyl group with respect to the hydroxyproline ring atoms in the above structure is very similar to that reported from the neutron diffraction studies on 4-hydroxyl-L-proline[2]. In fact, it is also possible to have the hydrogen bond from the Hyp OH to O_3 of a neighbouring protofibril through the intermediary of a water molecule. If this is made reasonably good with hydrogen bond lengths of the order of 2·75 A, then the separation between the neighbouring protofibrils comes to 14 Å. This roughly corresponds to the interprotofibrillar distance in collagen at normal humidities.

Thus, we see that Hyp can serve a double purpose in collagen—(*a*) it can form hydrogen bonds linking neighbouring peptide chains in a single triple helix and (*b*) it can also form hydrogen bonds with a different triple helix to link the two together. Thus, without ever forming a covalent linkage, hydroxyproline can serve a very important purpose in stabilizing the collagen structure. It may be mentioned that two recent studies have experimentally verified that the melting temperature, T^{m}, of collagen fibres is about 15° higher when the prolines in position 3 in the peptide chain are all hydroxylated, than when they are not hydroxylated[3,4]. In the work by Berg and Prockop[3], the T_m was measured directly from optical rotation studies and

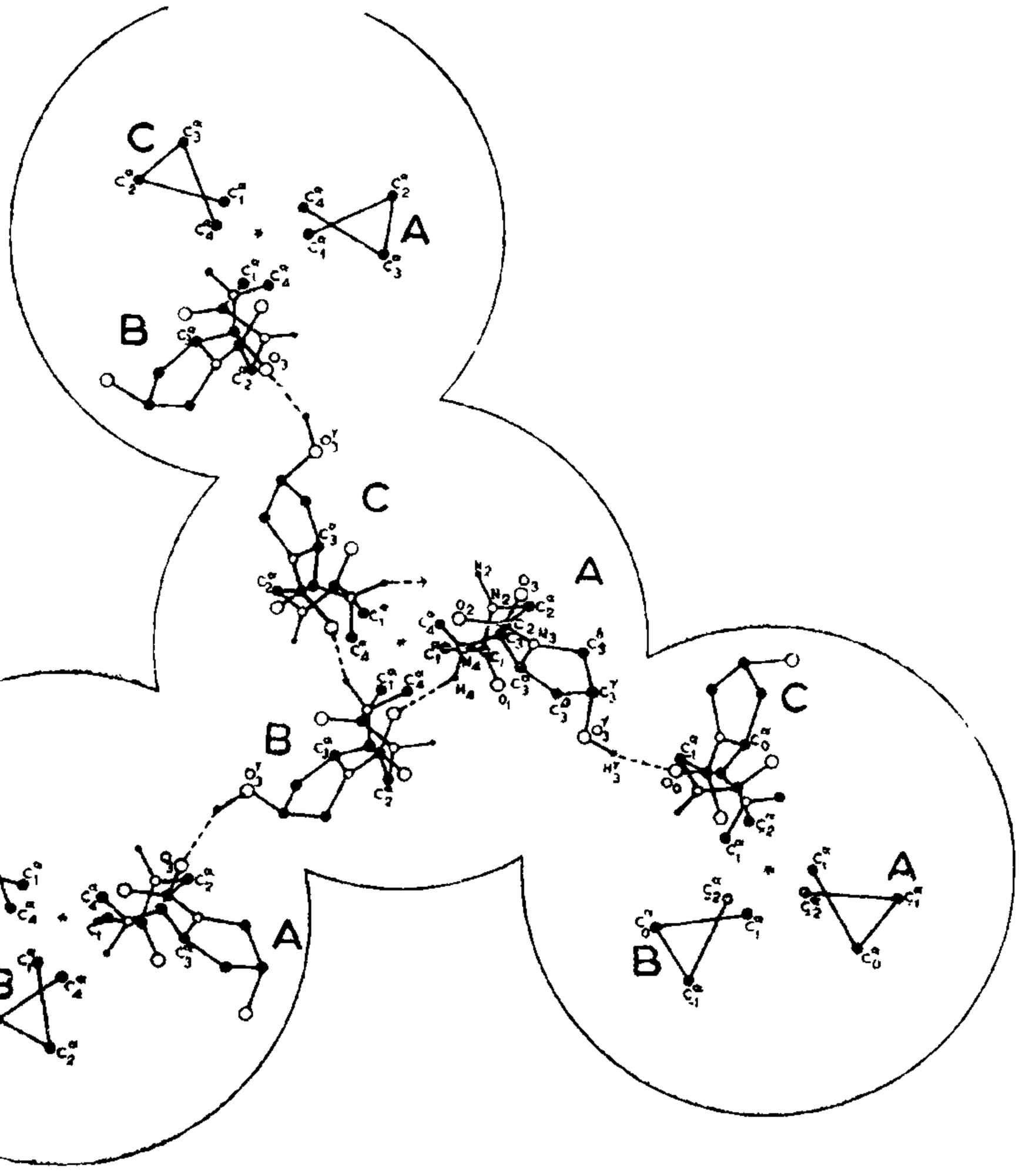

FIG. 2. The hydrogen bond scheme linking $O^{\gamma}H^{\gamma}$ of one protofibril in position 3 with O_3 of the next protofibril is shown for a central triple helix along with the three neighbouring protofibrils in hexagonal directions. It is to be noted that this linkage can be continued to form a good hexagonal latrice for the structure.

was shown to be appreciably larger for the hydroxylated form of collagen than the unhydroxylated form from the same source. In the latter studies by Jiminez *et al.*[4], the thermal stability of unhydroxylated collagen relative to hydroxylated collagen was investigated using pepsin digestion at various temperatures as an enzymatic probe of conformation. Their results also indicate that the unhydroxylated molecules have a denaturation temperature between 20° and 25°, while the hydroxylated molecules are stable beyond 35°. These studies can be taken to be very good evidence in support of the theoretical ideas put forward from our laboratory regarding the role played by hydroxyproline in the stability of the collagen molecule.

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