The Madras Group and the structure of collagen

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Abstract. The genesis of the collagen structure proposed by the Madras Group is briefly outlined. Recent crystal structure data on collagen-like peptides vindicate (i) the original proposal of Ramachandran and Kartha, namely, that the structure of collagen is a triple helical coiled-coil one, and (ii) the visualisation of the maximisation of hydrogen bonds directly or indirectly with water molecules, including C^{α}–H...O hydrogen bonds.

Keywords. Collagen; Madras Group; coiled-coil triple helix.

Collagen is biologically an extremely important molecule in its own right and represented the most complicated fibrous protein for structure elucidation at a time when the X-ray diffraction technique was being applied to resolve the three-dimensional architecture of biological macromolecules. In a matter of a few years between 1950 and 1960 major discoveries of macromolecular structural motifs, motifs that orchestrate a large number of protein and nucleic acid structures determined to date, were made in quick succession, fuelled by intuition and bold imagination and supported by X-ray diffraction patterns from fibres of these macromolecules. It is no exaggeration that this period was the Golden Era (the beginning) of structural molecular biology. Soon after the discovery of the α helix and β sheet structures by Linus Pauling (who became a role model for those involved in protein and nucleic acid structural research), there was a great flux of activity regarding the structure of collagen with a large number of people working on it and a number of conflicting models circulating in the field. More guesses on the structural models of collagen were made than on any other protein, and this was perhaps due to the more complex nature of collagen as compared to other fibrous proteins. It was into this state of affairs that Prof. G N Ramachandran entered the field propelled by a suggestion from Prof. J D Bernal during the latter's visit to Madras. Ramachandran took X-ray diffraction pictures of elastoidin (shark fin ray collagen) procured from the next-door Biochemistry Department of Madras University. Subsequently the neighbouring Central Leather Research Institute provided kangaroo tail tendon in abundance for X-ray diffraction analysis of collagen and thus played a crucial role in the proposal of the triple helical model.

It is now acknowledged that the first correct model for the structure of collagen was provided by Ramachandran and Kartha[1]. This striking structure consisted of three parallel polypeptide chains related by three-fold symmetry and held together by hydrogen bonds.

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Each chain had 3_2 symmetry. Though this structure explained the features of the infrared spectrum and the chemical composition of collagen, it was not consistent with the X-ray data of *stretched* collagen. The model demanded that the prominent 2.86 Å meridional reflection was an unresolved pair of non-meridional reflection. But the X-ray diffraction patterns obtained by Cowan, North and Randall[2] and by Ramachandran and Ambady[3] (figure 1) of stretched collagen and stretched and tilted collagen respectively showed that the 2.86 Å reflection was in fact truly meridional. It was also shown that all the layer lines could be indexed on the basis of ten residues per three turns with a residue height of 2.95 Å.

Incorporation of these features obtained from the X-ray diffraction pattern of collagen (namely, the number of repeating units is around the nonintegral value 3.3 instead of the integral 3, and the truly meridional nature of the 2.86 Å reflection) demanded some modification in the triple stranded structure. This was successfully tackled by Ramachandran and Kartha[4] by introducing a coiling of the three chains around a common axis visualising the structure of collagen to be a coiled-coil, a concept earlier introduced by Crick for the packing of α-helices. This coiled-coil structure nevertheless retained all the essential features of the original Ramachandran–Kartha model as regards the location of amino-acid residues and the orientation of NH and CO bonds (figure 2). The two hydrogen bonds of the structure, in a sequence (Gly–X–Y), corresponded to (i) Gly N–H hydrogen bonded to C=O of the Y residue, and (ii) N–H group of X residue, when it was not an imino acid, hydrogen bonded with C=O of X of the neighbouring chains. In
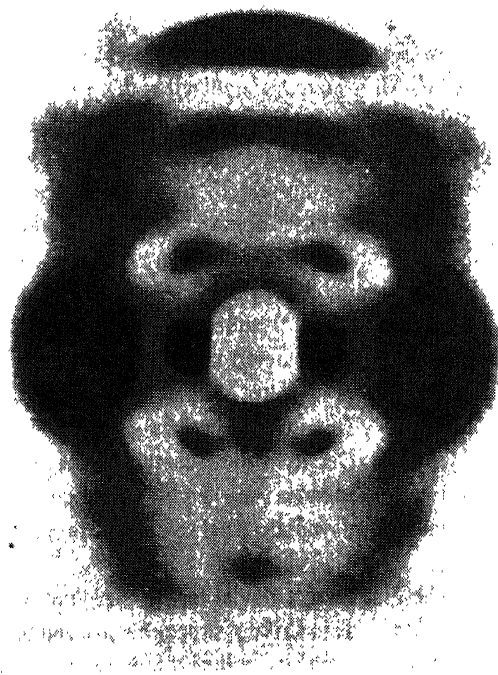

Figure 1. X-ray diffraction pattern of stretched collagen fibre kept at 75° to the X-ray beam (Ramachandran and Ambady[3]).

addition, C^{α}-H...O hydrogen bonds were also proposed subsequently for the first time for collagen by Ramachandran and Sasisekharan[5] and visualized recently in the crystal structures of collagen-like peptides determined by Berman, Brodsky and their colleagues[6-8]. The structure with two N-H...O hydrogen bonds could also explain the occurrence of a fraction of more than one-third glycine residues and readily accommodate proline and hydroxyproline residues. A structure with (Gly-Pro-Hypro) sequence would of course have only one hydrogen bond between the chains for every three residues.

The international scientific community, especially the West, perhaps because of the difficulty in pronouncing the names of Prof. Ramachandran and his associates from the then Physics Department of Madras University identified them as the *Madras Group* while referring to their contributions, and designated the triple helical structure of
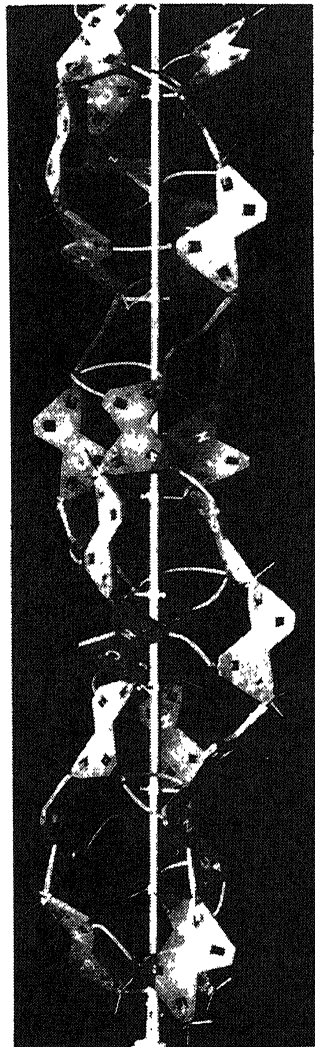

Figure 2a. Triple-helical structural model of collagen made of planar peptide units and constructed in the Department of Physics, Madras University by Prof. Ramachandran.
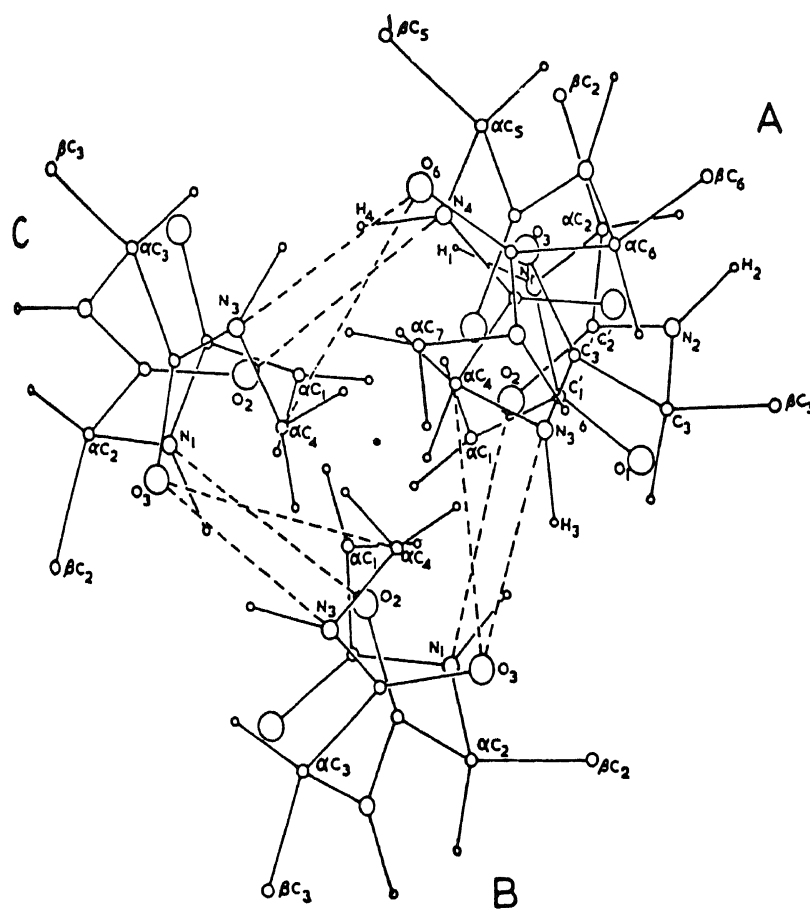
Figure 2b. Projection of collagen structure down the helical axis for a height of three residues. N–H...O hydrogen bonds for every three residues for the sequence Gly–X–Y along with the possibility of C^{α}–H...O hydrogen bonds (Ramachandran and Sasisekharan[5]) are shown.

collagen discovered by them as the *Madras helix*. In the Indian context it is indeed a matter of greatest pride that the Madras group headed by Prof. Ramachandran was one of a few elite bands of scientists engaged in tackling the most challenging and contemporary research subject to discover the three-dimensional structures of biological macromolecules and their folding principles, a problem that currently goes under the fashionable name of "Structural Biology". Those were the days when the "alphabets" of protein and nucleic acid structures were being hotly pursued by young scientists who subsequently became famous. The Madras Group was nascent, isolated and had no previous history of scientific research in this area of biological structures, compared to the English or the California groups who were pioneers in this field of science. Besides Prof. G N Ramachandran and Dr Gopinath Kartha, the other scientific collaborators of Prof. Ramachandran on the research project on collagen structure at that time included G K Ambady, V Sasisekharan, Y T Thathachari, B R Lakshmanan and C Ramakrishnan. Now all this has changed including the name of the city of Madras (now Chennai).

The two N–H...O hydrogen-bonded structure of Ramachandran–Kartha tacitly complied with the then prevailing dogma (consequent to the discovery of fully hydrogen-bonded α helix and β strand motifs) that wherever possible N–H and C=O groups should necessarily be engaged to maximise hydrogen-bond interactions. Subsequently, however, Rich and Crick[9] proposed a single hydrogen-bonded version of the Ramachandran–Kartha triple helical coiled-coil structure of collagen. They contended that throughout the structure of collagen, irrespective of whether the sequence contains (Gly–Pro–Hypro) or not, there can be only one N–H...O hydrogen bond for every three residues and not two as postulated by the Madras Group. They argued that a triple-stranded coiled-coil structure cannot be built for collagen with two hydrogen bonds for every three residues without certain "unfavorable" nonbonded van der Waal's contacts. Rich and Crick[10] felt that the Madras triple helix was *not correct but of the correct type* and did not favour it on *aesthetic grounds*. About the same time a similar structure with one hydrogen bond for every three residues was proposed by Cowan, McGavin and North[11]. Subsequently refinement of the collagen structures with one hydrogen bond for every three residues was reported by Fraser and coworkers[12].

Incidentally, it was also found by the Madras group from a systematic and a thorough examination that it was impossible to build a two-hydrogen-bonded structure with a few interatomic non-bonded distances greater than the conventional van der Waal's separations by using perfectly *planar peptide units with rigid geometry*[13,14] (see *Addendum*). There were no hard and fast criteria available in the literature for judging a structure to be good or bad during those days. A systematic survey of non-bonded separations from crystal structures carried out by the Madras group showed that the so-called "bad contacts" in the two N–H...O bonded triple helical structure turned out to be after all "no bad contacts". In fact Linus Pauling had mentioned quite early in his celebrated book *'Nature of the chemical bond'* that the equilibrium distance between pairs of non-bonded atoms could be less by as much as 11% of the sum of their van der Waal's radii in the solid state. It is now history as to how these criticism of "bad contacts" in the triple helical structure of collagen turned out to be a blessing in disguise for the Madras group, as it laid the foundation for another breakthrough in quick succession, through the now well-known *Ramachandran map*. The principles of this were to have an impressive and deep impact on the development of computational methods for conformational analysis of not only proteins but also other biopolymeric systems. Linus Pauling during his visit to Madras in the year 1967 complimented the young scientists for their independent discovery of the equilibrium distance of non-bonded atoms (crushed nature) in the solid state through their analysis of the then available crystal data. He wondered why this was not recognised or taken note of by others. The observations of the Madras group demonstrated that it was not correct to criticise the two-bonded structure of collagen with two N–H...O hydrogen bonds for every three residues as too impossibly crushed, since the non-bonded interaction energy was not unfavorable for it. On the other hand, collagen structure with two hydrogen bonds would definitely be expected to be more stable than the one-bonded structure.

Collagen structure being what it is, one has to reckon with the major structural feature, namely, the way in which each of the three polypeptide chains with an approximate three-fold symmetry fold into left handed helices, and how these undergo further supercoiling in a right handed fashion about a common axis to form a coiled-coil. As pointed out long ago polypeptide chains in the collagen structure have a natural conformation; that is, one which they would have even in the absence of hydrogen bonds that stabilise the triple

chain conformation further[15]. This renders the question of whether there is one hydrogen bond or two hydrogen bonds (directly involving the functional groups) for every three residues between the chains a 'non issue'*. After all hydrogen bonds could be formed either directly or through water involving the functional groups.

The Madras Group also worked out a triple stranded structure having only one hydrogen bond, Gly N–H with (X) C=O proposed in the sequence $(Gly-X-Y)_n$ for every three residues satisfying the conventional and the then prevailing ideas about the ideal van der Waal's separation between non-bonded atoms. The co-ordinates of this structure obtained by an analytical approach (see addendum) during 1957–58 are given in the Ph D thesis[14] of Sasisekharan. They further found that several models could be built for the structure of collagen with one hydrogen bond for every three residues with essentially similar conformational features. Imposition of the condition that as many of the functional groups as possible should be hydrogen bonded (in other words maximisation of the hydrogen bonds in the structure for enhancing the stability of collagen structure) led to the suggestion[13] that further stabilistion of the one hydrogen-bonded collagen structure could be achieved through water-mediated hydrogen bonds. Such a structure of collagen with hydrogen bonds through water molecule was proposed by Ramachandran and Chandrasekharan[16] (figure 3). It is noteworthy that these features about the role of water
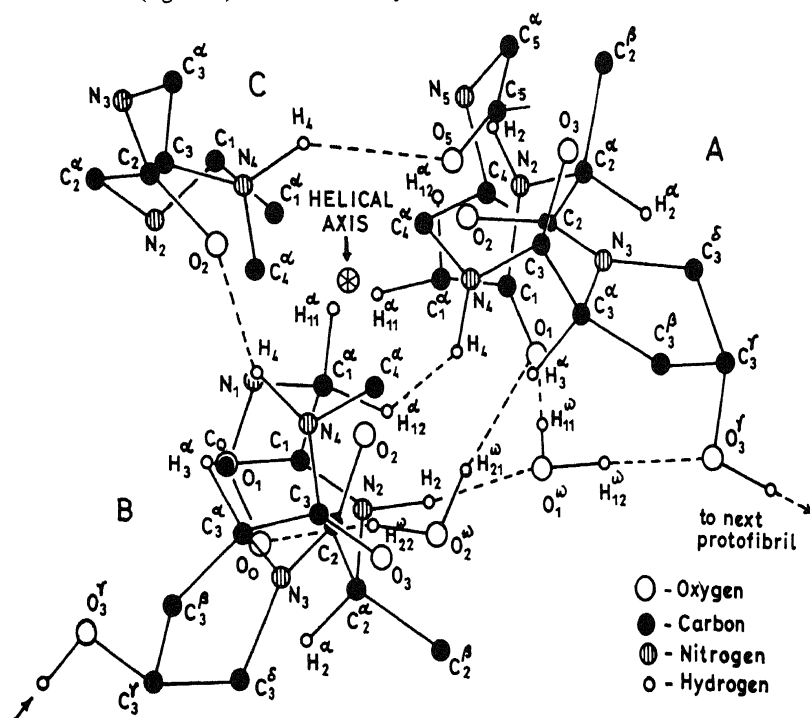

Figure 3. Projection down the helical axis of atoms of the collagen triple helix. The interchain hydrogen bonds and also the interaction of two water molecules bound to the chains A and B are indicated (from the text of Gandhi Memorial Lecture delivered by Prof. Ramachandran at the Raman Research Institute, Bangalore on October 2, 1975).

*After having described an animal sighted in the jungle, with all descriptions pointing to its being an elephant, it is pointless to argue that no mention was made whether the elephant had one tusk or two tusks, meaning thereby that if it had one tusk, it could be a rhinoceros.

as well as the scheme of C^{α}-H...O hydrogen bond interactions proposed by the Madras Group are well-manifested in the recent crystal structures of collagen-like oligopeptides containing (Gly–Pro–Hypro) and (Gly–Pro–Pro) triplet sequences[6–8].

Only about 10% of the triplets seemingly constitute the sequence (Gly–Pro–Hypro) in collagen and this triplet sequence necessarily can form only one hydrogen bond. In the remaining 90% of the other triplet sequences, it is entirely possible that triple-helical structures with one hydrogen bond and with many of the functional groups involved in water-mediated hydrogen bonding or with two hydrogen-bonded conformations and/or with side chain... side chain interactions with or without metal ions could as well prevail. Further, helical parameters quite different from the value expected from fiber (10/3), such as 3·5 residues per turn (7/2) with a corresponding value for twist angle are also found in crystal structures[6–8]. Thus, in addition to maximisation of hydrogen bonding interactions, other generic models possessing different helical parameters bearing e.g. the symmetries 17/5, and 27/8, in addition to the 7/2 and 10/3 already seen (symmetry series for structures of the collagen types appears to be 4/1, 3/1, 7/2, 10/3, 17/5, 27/8, 44/13, 71/21 etc., in contrast to the series of α-helix types, viz. 3/1, 4/1, 7/2, 11/3, 18/5, 29/8, 47/3, etc.), might also occur at specific triplet sequences along the chain, as more structures of collagen-like peptides are determined from single crystal studies.

Addendum

While this note was being prepared the nostalgic narration of personal nature by Dr Rich on the discovery of collagen structure[17] came to our attention. The Madras Group of workers and especially one of the present authors (VS) built a large number of models for triple-stranded collagen with peptide charts generated using the cyclographic projection technique[14], an analytical approach. This had a built-in feature to incorporate small variations in bond angles, bond lengths and nonplanar distortions in peptides, compared to the rigid Kendrew or CPK models. Besides, it had the advantange of measuring co-ordinates in a much more sophisticated and accurate way than one would obtain from construction of physical wire models and using plumbline or light shadow methods. Very small distortions in the peptide geometry were found necessary to build a two-hydrogen-bonded triple-stranded structure of collagen, in order to maximise direct hydrogen bonding interactions between functional groups and hence enhance stabilising interactions. Such small distortions were justified as they were found in the crystal structures of peptides. The present day computer modellers can readily appreciate such distortions. It is not therefore surprising that Rich and Crick landed up with a structure with one hydrogen bond for every three residues using peptides with rigid geometry. Based on the observations from the crystal structures of collagen-like peptides[6–8] comprising (Gly–Pro–Hypro) and (Gly–Pro–Pro) triplets, Rich now feels that their proposal for the structure of collagen which has one hydrogen bond for every three residues is *the structure* of collagen. In one sentence, he dismissed the structure proposed by Ramachandran and his group as "wrong". In the context of the history and entirety of the collagen structure, to pronounce a judgement on the correct or incorrect nature of the collagen structure based on the above fact alone without a holistic consideration reflects reluctance to bestow due credit to the original and first discoverers who enunciated the basic tenets of the collagen structure.

On the other hand, crystal structure data vindicate not only the structure proposed by the Madras Group for collagen, namely, that it has triple-stranded coiled-coil structure but

also the correctness of the collagen structure on the maximisation of hydrogen bonds, directly or indirectly with water molecules, including the C^α–H...O hydrogen bonds. In any case, as stated earlier, several of the variations in collagen structure are derivable from the generic triple-stranded coiled-coil structure of Ramachandran and Kartha.

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