Axial arrangement of α-chains in the collagen molecule: intramolecular polar and apolar interactions

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Polar and apolar intramolecular interactions have been calculated for the type I collagen molecule. These interactions were determined as a function of the relative axial stagger between the three α-chains (two α_1 and one α_2). The results clearly show that a relative stagger of only one residue translation (2.86 Å) between adjacent chains is optimal. The criteria chosen to define the polar and apolar interactions do not distinguish between the three possible ways in which three α-chains in the collagen molecule can be linearly arranged i.e. $\alpha_1\alpha_1\alpha_2$, $\alpha_1\alpha_2\alpha_1$ and $\alpha_2\alpha_1\alpha_1$. It appears probable that this information lies mainly in the non-helical portions of the procollagen molecule.

INTRODUCTION

The predominant type of collagen from the higher animals consist of three α-chains, two of which are identical (α_1) and one of which is different (α_2) (ref 1). The sequence of the α_1-chain of rat/calf skin collagen consists of 1052 residues[2], 1011 of which are arranged in triplets. Position 1 in each triplet is Gly whilst positions 2 and 3 are occupied by residues X and Y respectively. Certain residues such as Pro, Leu, Phe and Glu fall predominately into the X category and other residues such as Hyp and Arg fall mainly into the Y category. The triplet portion of the α-chains of collagen can thus be summarized as $(Gly\text{-}X\text{-}Y)_{337}$. This section of the two α_1-chains and the one α_2-chain combine to form the triple helical region of the collagen molecule[3,4]. The N-termini of all three chains lie at the same end of the molecule. In addition, there are short regions known as telopeptides at the N- and C-terminal ends of the α-chains which do not have a triplet structure. These are structurally important since they contain the lysine residues involved in the formation of intra- and intermolecular covalent crosslinks[5]. Hulmes *et al.*[2] were able to show that if the homologous α_1 and α_2-chains were assumed to be identical[5,6] and the sequence of the triplet region was analysed as if the collagen molecule was linear, then the total number of polar and apolar interactions between molecules peaked at relative axial staggers of 0*D*, 1*D*, 2*D*, 3*D* and 4*D* where $D = 234 \pm 1$ residue translations. A stagger of *D* (or a multiple) between adjacent molecules is consistent with that proposed by Hodge[7] from electron microscope observations on the axial period (~600–670 Å) of the negatively stained banding patterns of native fibrils and also with the low-angle X-ray diffraction patterns of collagen which show meridional reflections indexing on a period of 635–670 Å (depending on the state of hydration). It is the intention of this paper to discuss the nature of the intramolecular interactions which may be important in stabilizing the axial arrangement of two α_1-chains and one α_2-chain.

APOLAR AND POLAR INTERACTIONS

Reconstitution studies have shown that three α_1-chains form a stable triple helix[8]. Using this as a test case, various types of stabilizing interactions may be proposed and their number calculated. The results may be compared for statistical significance with the mean number of interactions found using a set of 200 'random α_1-sequences'. Each of these 'random sequences' has as its starting point the known triplet region of the α_1-sequence of rat/calf skin collagen. Certain restrictions were applied to the randomization procedures employed. Firstly, the Gly residues in position 1 were left unchanged. Secondly, residue X in a randomly chosen triplet may be exchanged only with residue X in another randomly chosen triplet. Similarly, a residue Y may be exchanged with another residue Y on the same basis. Procedures of this type are required in order to maintain the amino-acid compositions of positions 2 and 3 in the triplet since, as has been stated previously, some amino-acids are not uniformly distributed between positions 2 and 3. The triplets containing either of the X (or Y) residues to be exchanged were chosen using a random number generator routine. Almost 30 000 random exchanges were performed computationally before a final 'random sequence' was obtained. The whole procedure was then repeated 200 times to produce a library of random sequences.

It seems likely that the Rich–Crick Collagen II triple helix (one-bonded) is the basic conformation in collagen[5] and inspection of this model shows that on the surface of the molecule pairs of closely spaced amino-acid side chains occur at axial intervals of one residue translation (2.86 Å). Each pair comprises a residue in position 2 from one α-chain and a residue in position 3 from another α-chain. When these residues are both imino-acids (Pro, Hyp), the planes of the pyrollidine rings are approximately parallel to one another. Since the repeating tripeptide $(Gly\text{–}Pro\text{–}Pro)_n$ has been shown by both physical and chemical methods to form a stable triple helical structure very much like that observed

in collagen, it would appear that the interactions between pyrollidine rings may be a stabilizing influence on the tertiary structure. Furthermore the interactions between α-chains are likely to be very similar to those suggested by Hulmes *et al.*[2] since their choice of criteria led to the observed experimental data.

Proceeding from the N-terminal end of the molecule, chain 2 is axially staggered by m residue translations with respect to chain 1 and chain 3 is axially staggered by n residue translations with respect to chain 2. Both m and n are positive integers greater or equal to unity. It follows from the conformation of the triple helical structure that m, n or $m + n$ cannot be a multiple of 3. Apolar and polar interactions of unit weight were defined respectively as the coincidence at any of the $1011 - m - n$ 'levels' along the three chain triplet region of (a) two of Pro, Hyp, Leu, Phe, Met, Ile and Val and (b) either Asp or Glu with Lys or Arg. Unsatisfactory polar interactions were also calculated where these refer to the coincidence (as defined above) of similarly charged residues, e.g. Asp and Glu or Lys and Lys. Although the possible number of stabilizing interactions between the three α-chains decreases as m and n increase, no allowance has been made to compensate this small effect (i.e. a possible drop of 1–2% in the number of interactions). Indeed, it is imperative that no such compensation be made since it is the *absolute* number of interactions that will be important in determining the relative chain stagger and not the number of interactions per residue. Interactions of unit weight are an over-simplification since the side chains of several amino-acids, such as lysine, exhibit both apolar and polar features and certain combinations of amino- and imino-acids must be more significant than others.

The definition of apolar interactions used in this work differs from that used previously[2] only in the present inclusion of Pro and Hyp in the list of interacting apolar residues. The rationale for this addition, has in part, been explained earlier in the text but in addition, the omission of Pro and Hyp in the earlier work on the intermolecular interactions was based on the premise that the 'side chains' of Pro and Hyp would not be long enough to interact frequently with other apolar residues. However, in the present work, the intramolecular interactions occur between chains wound about a common axis and the side chains are automatically in close contact. The length of the side chains becomes less important in this situation.

RESULTS

Table 1 shows the number of interactions for the $\alpha_1\alpha_1\alpha_1$ model (Model 1) with m and n = 1,2,4,5,7, or 8 residue translations. When $m = n = 1$ residue translation, the apolar interactions are 2.2 standard deviations above norm and the 'good' and 'bad' polar interactions are 5.4 and 2.2 standard deviations above and below norm respectively, where norm refers to the mean number of interactions found for the 200 α_1-sequences randomized as described previously. Statistically this result is the most significant of the possibilities considered. It can be argued that the types of interactions used in the calculations are reasonably realistic in that their use has led to the prediction of one and only one arrangement of three α_1-chains that has significantly more stabilizing intramolecular interactions than any other.

A similar procedure can be applied to models containing two α_1 and one α_2-chains. There are three ways (*Figure 1*) in which these three similarly directed α-chains can be arranged starting from the N-terminal ends namely $\alpha_1\alpha_1\alpha_2$ (Model 2), $\alpha_1\alpha_2\alpha_1$ (Model 3) and $\alpha_2\alpha_1\alpha_1$ (Model 4). Even though it is likely that m equals n, the interactions for all eighteen possible combinations of m and n (see *Table 1*) have been calculated, based upon the following assumptions. (a) The unknown portion of the α_2-sequence is identical to the corresponding part of the α_1-chain. About 65% of the α_2-sequence is currently at hand[9–12]. (b) The α_2-sequence contains a triplet region of the same length as the α_1-sequence[12]. (c) The maximum value of m or n is eight residue translations. Doyle *et al.*[13] have shown that the positively stained banding pattern of rat tail tendon observed in the electron microscope may be produced directly from the sequence by staggering three α_1-chains by one residue with respect to one another. The positively-stained α_2 SLS pattern is almost identical to the α_1 SLS pattern which justifies this approach. However, the resolution in the electron micrographs is only about 20 Å and an equally satisfactory fit with the electron micrographs could be obtained by staggering the α-chains axially with respect to one another by a few residue translations. The value of eight residue translations chosen is the maximum possible based on the resolution of the micrographs.

This method of calculating the stabilizing interactions of the collagen molecule should not be taken to indicate that the contribution to the ordering of the α-chains is thought to lie entirely in the triplet region of the sequence. The telopeptides may have an important contribution to play though the uncertain geometry of these portions of the polypeptide chain make the role less easily determinable.

It is not surprising that (from *Table 1*) the case when $m = n = 1$ residue translation again has a significantly higher number of interactions than that for any other arrangement. For Models 2, 3 and 4 the apolar interactions are about 2.0–2.5 standard deviations above norm and the good and bad polar interactions 5.1–6.1 and 2.0–2.6 standard deviations above and below norm respectively. The level of significance has, in general, slightly increased relative to Model 1. Again, norm refers to the mean number of interactions for the 200 'random' sequences generated as described earlier. In addition, the α_1 and α_2-sequences were randomized in such a way as to preserve homology between the α_1 and α_2-chains. i.e. the randomly chosen triplets between which there is an exchange of residues are identical for the 'random α_1-sequence' and the 'random α_2-sequence'. All other collagen molecules, with the probable exception of that from cod fish[14] consist of three identical α-chains. As the relative stagger between three identical chains is likely to be the same, the results presented here suggest that α-chains of any type preferentially stagger axially with respect to one another by one residue translation. Although substitution in positions X and Y between the α_1 and α_2-chains is about 50%[15], there is a smaller percentage rate of substitution in the types of residues considered to be forming the intramolecular interactions. As defined in this work, the apolar (Pro, Hyp, Leu, Met, Phe, Ile, Val), basic (Lys, Arg) and acidic residues (Glu, Asp) in the α_1-sequence are 80, 85 and 70% conservatively retained in the α_2-sequence. The unknown interactions, due to the incomplete α_2-sequence, will change the level of significance of the result but the conclusion that there is one residue axial stagger between chains is unlikely to change.

From this work it is not yet possible to choose between Models 2, 3 and 4 though it had been suggested from a limited analysis of potential interactions[15] that Model 3 was

Table 1 Intramolecular interactions as a function of relative chain stagger. For each value of *m* and *n* two polar interactions are listed. The first value refers to 'satisfactory' polar interactions, i.e. between side chains of opposite charge such as Lys and Glu, whilst the second value refers to 'unsatisfactory' polar interactions, i.e. between side chains of similar charge such as Lys and Arg or Glu and Glu. The columns headed 'real' refer to the intramolecular interactions between the known α_1 and/or α_2-sequences and the columns headed 'random' refer to intramolecular interactions between the randomized α_1 and/or α_2-sequences

	Stagger		Model 1		Model 2		Model 3		Model 4		Model 5	
	m	*n*	Real	Random ± SD	Real	Random ± SD	Real	Random ± SD	Real	Random ± SD	Real	Random ± SD
Apolar			213	190.5 ± 10.4	222	199.9 ± 9.7	222	199.1 ± 9.3	220	199.3 ± 9.9	239	218.9 ± 10.3
Polar	1	1	74	38.5 ± 6.6	68	35.9 ± 6.3	73	35.9 ± 6.1	77	36.2 ± 6.6	67	31.3 ± 6.4
Polar			−12	−23.8 ± 5.5	−11	−22.0 ± 5.3	−11	−22.0 ± 5.0	−9	−22.0 ± 5.0	−7	−18.7 ± 4.9
Apolar			198	189.1 ± 10.5	203	198.4 ± 10.3	205	198.7 ± 8.8	201	198.2 ± 9.1	223	218.6 ± 10.1
Polar	2	2	59	37.7 ± 6.3	56	35.5 ± 6.3	57	35.5 ± 6.2	59	35.5 ± 6.2	53	31.2 ± 6.7
Polar			−16	−23.5 ± 5.4	−17	−21.7 ± 5.2	−13	−21.7 ± 4.9	−11	−21.7 ± 5.2	−8	−18.5 ± 4.9
Apolar			179	190.1 ± 9.6	187	199.1 ± 8.9	180	199.1 ± 8.5	190	198.7 ± 9.3	204	217.7 ± 9.7
Polar	4	4	21	37.6 ± 5.8	23	35.4 ± 5.4	20	35.5 ± 5.5	21	35.3 ± 5.8	23	31.0 ± 5.7
Polar			−27	−23.2 ± 5.5	−26	−21.5 ± 5.3	−26	−21.5 ± 4.9	−26	−21.4 ± 5.2	−22	−18.1 ± 5.1
Apolar			184	188.6 ± 9.8	196	198.1 ± 9.6	192	198.9 ± 8.9	195	198.5 ± 8.7	225	218.7 ± 9.0
Polar	5	5	47	37.6 ± 6.7	46	35.4 ± 6.4	44	35.2 ± 6.2	45	35.3 ± 6.3	43	30.8 ± 6.0
Polar			−11	−22.8 ± 5.7	−12	−21.1 ± 5.4	−12	−21.1 ± 5.3	−9	−21.1 ± 5.3	−9	−17.9 ± 5.1
Apolar			191	189.8 ± 10.1	201	199.8 ± 10.2	198	198.8 ± 9.0	194	198.3 ± 9.2	215	217.1 ± 10.5
Polar	7	7	38	37.2 ± 6.7	35	35.0 ± 6.3	35	35.0 ± 6.1	33	35.0 ± 6.4	28	30.9 ± 5.6
Polar			−16	−22.9 ± 5.8	−16	−21.2 ± 5.6	−16	−21.2 ± 5.4	−15	−21.1 ± 5.5	−15	−18.0 ± 5.1
Apolar			160	189.3 ± 10.4	176	198.2 ± 9.5	178	198.0 ± 9.5	175	197.0 ± 10.0	203	212.1 ± 9.7
Polar	8	8	23	37.5 ± 6.7	25	35.1 ± 6.1	26	35.1 ± 6.1	24	35.1 ± 6.3	27	35.7 ± 5.8
Polar			−32	−22.9 ± 5.5	−30	−21.0 ± 5.4	−31	−21.0 ± 5.4	−32	−21.3 ± 5.2	−29	−18.1 ± 5.5
Apolar			196	190.3 ± 8.1	198	199.8 ± 7.5	201	199.2 ± 8.0	207	199.7 ± 8.2	222	219.4 ± 8.1
Polar	1	4	49	38.2 ± 4.8	48	35.9 ± 4.6	45	35.7 ± 4.9	49	35.7 ± 4.9	45	31.1 ± 4.7
Polar			−13	−23.3 ± 4.1	−15	−21.6 ± 4.0	−10	−21.5 ± 4.1	−13	−21.6 ± 4.0	−10	−18.3 ± 4.0
Apolar			195	191.0 ± 7.9	209	200.6 ± 8.5	200	199.3 ± 8.2	210	199.3 ± 7.7	226	217.8 ± 8.2
Polar	1	7	44	37.9 ± 4.9	46	35.6 ± 4.7	38	35.4 ± 4.8	47	35.5 ± 4.9	41	31.0 ± 4.6
Polar			−20	−23.4 ± 4.2	−18	−21.5 ± 4.1	−19	−21.7 ± 4.2	−21	−21.6 ± 4.2	−18	−18.3 ± 4.0
Apolar			197	189.8 ± 7.7	205	199.9 ± 7.8	208	199.9 ± 7.9	200	198.3 ± 7.6	228	219.1 ± 7.4
Polar	2	5	57	37.6 ± 5.1	56	35.4 ± 4.7	52	35.2 ± 4.9	55	35.5 ± 5.1	49	31.0 ± 4.6
Polar			−10	−23.2 ± 4.6	−11	−21.4 ± 4.6	−10	−21.4 ± 4.4	−7	−21.5 ± 4.3	−7	−18.2 ± 4.1
Apolar			184	188.6 ± 7.8	202	197.6 ± 8.1	199	197.6 ± 8.0	191	198.0 ± 7.8	224	216.6 ± 7.6
Polar	2	8	44	37.4 ± 5.1	46	35.2 ± 5.0	42	35.0 ± 5.1	45	35.3 ± 5.0	43	30.8 ± 4.9
Polar			−24	−23.1 ± 4.5	−22	−21.3 ± 4.3	−25	−21.3 ± 4.5	−19	−21.5 ± 4.7	−18	−18.1 ± 3.9
Apolar			196	190.2 ± 8.2	202	199.8 ± 7.8	201	198.8 ± 7.9	203	199.9 ± 8.3	222	219.3 ± 8.1
Polar	4	1	49	38.2 ± 4.8	45	35.6 ± 4.6	51	35.9 ± 4.9	46	35.8 ± 4.9	45	31.1 ± 4.8
Polar			−13	−23.3 ± 4.0	−13	−21.7 ± 4.0	−14	−21.6 ± 4.0	−11	−21.5 ± 4.0	−10	−18.4 ± 4.0
Apolar			194	189.7 ± 8.2	204	199.2 ± 8.5	195	198.0 ± 7.7	203	198.6 ± 8.2	215	217.0 ± 7.8
Polar	4	7	33	37.5 ± 5.2	30	35.2 ± 4.8	30	35.3 ± 4.8	33	35.2 ± 5.3	28	31.0 ± 4.5
Polar			−18	−23.2 ± 4.6	−18	−21.2 ± 4.4	−19	−21.5 ± 4.2	−15	−21.3 ± 4.5	−16	−18.1 ± 3.9
Apolar			197	189.8 ± 7.8	208	199.7 ± 8.1	201	199.6 ± 7.6	204	198.9 ± 7.6	228	219.2 ± 7.3
Polar	5	2	57	37.6 ± 5.1	54	35.4 ± 4.8	57	35.3 ± 5.0	52	35.2 ± 4.9	49	31.0 ± 4.6
Polar			−10	−23.2 ± 4.6	−10	−21.3 ± 4.3	−8	−21.6 ± 4.6	−10	−21.5 ± 4.4	−7	−18.3 ± 4.1
Apolar			170	189.3 ± 7.9	188	198.1 ± 8.1	182	198.6 ± 7.9	179	198.4 ± 7.8	211	217.0 ± 8.1
Polar	5	8	42	37.4 ± 5.2	45	35.0 ± 4.9	42	35.0 ± 4.8	40	35.1 ± 5.1	43	30.6 ± 4.6
Polar			−15	−22.9 ± 4.4	−12	−21.1 ± 4.5	−17	−21.2 ± 4.3	−16	−21.1 ± 4.3	−14	−18.0 ± 4.2
Apolar			194	191.0 ± 7.9	206	200.0 ± 8.2	205	200.1 ± 8.0	206	199.1 ± 8.1	226	217.8 ± 8.1
Polar	7	1	44	37.9 ± 5.0	43	35.4 ± 4.8	46	35.7 ± 4.8	42	35.6 ± 4.8	41	31.0 ± 4.6
Polar			−20	−23.4 ± 4.2	−17	−21.6 ± 4.2	−20	−21.6 ± 3.9	−21	−21.6 ± 4.2	−18	−18.4 ± 4.0
Apolar			193	189.8 ± 8.1	197	198.6 ± 8.2	200	199.3 ± 8.4	203	198.2 ± 8.1	215	217.0 ± 7.7
Polar	7	4	33	37.5 ± 5.2	30	35.2 ± 4.9	32	35.2 ± 5.0	31	35.1 ± 5.1	28	30.9 ± 4.5
Polar			−18	−23.2 ± 4.6	−19	−21.4 ± 4.4	−16	−21.3 ± 4.3	−17	−21.5 ± 4.4	−16	−18.1 ± 3.9
Apolar			183	188.5 ± 7.9	198	197.8 ± 8.1	193	197.8 ± 8.2	199	197.5 ± 8.2	224	216.6 ± 7.6
Polar	8	2	44	37.4 ± 5.1	41	35.2 ± 5.2	47	35.1 ± 5.2	45	35.1 ± 4.9	43	30.8 ± 4.9
Polar			−24	−23.1 ± 4.5	−24	−21.4 ± 4.5	−19	−21.3 ± 4.3	−23	−21.4 ± 4.4	−18	−18.1 ± 4.0
Apolar			169	189.3 ± 7.8	181	198.6 ± 7.9	183	199.1 ± 7.9	183	197.3 ± 7.5	211	216.9 ± 8.1
Polar	8	5	42	37.4 ± 5.2	42	35.0 ± 4.9	42	35.0 ± 4.9	43	35.2 ± 5.1	43	30.6 ± 4.7
Polar			−15	−22.9 ± 4.4	−15	−21.3 ± 4.3	−13	−21.0 ± 4.5	−17	−21.1 ± 4.3	−14	−18.0 ± 4.1

Model 1: $\alpha_1\alpha_1\alpha_1$; Model 2: $\alpha_1\alpha_1\alpha_2$; Model 3: $\alpha_1\alpha_2\alpha_1$; Model 4: $\alpha_2\alpha_1\alpha_1$; Model 5: $\alpha_2\alpha_2\alpha_2$.

most likely to be correct. It is known that the thermal stability of Model 1 should be similar to that of Models 2, 3 or 4 and that both of these models should have a greater stability than Model 5. Model 5 consists of three α_2-chains. *Table 1* does indeed indicate that Model 1 will have similar stability to Models 2, 3 and 4. Model 5 has more apolar interactions and less polar interactions than the other models and will only be less stable on the criteria chosen if the polar interactions are weighted more heavily than the apolar interactions.

Clearly the differences between the triplet portions of the α_1 and α_2-sequences are not sufficiently pronounced for the axial order (as distinct from axial stagger) of the two α_1 and one α_2-chain to be uniquely determined from a study of this type. It would appear that the telopeptides and/or procollagen extensions have a crucial role in the ordering of the α-strands in the triple helix but once the latter has been formed (with a relative stagger of one residue translation, 2.86 Å, between chains), the collagen molecule will be in a highly favourable energetic state.

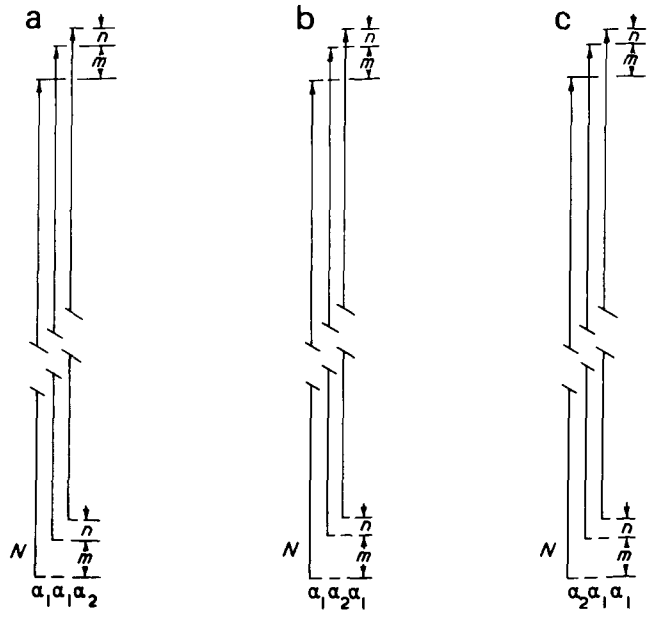

Figure 1 The axial stagger of *m* and *n* residue translations between the first and second chains and between the second and third chains respectively is illustrated for (a) Model 2 ($\alpha_1\alpha_1\alpha_2$), (b) Model 3 ($\alpha_1\alpha_2\alpha_1$) and (c) Model 4 ($\alpha_2\alpha_1\alpha_1$). The α-chains in these models are ordered consecutively according to the proximity of the N-terminal end of the collagen molecule to the first Gly in the triplet portion of each α-chain

The sequence (Gly–imino–imino)$_5$, which terminates the carboxyl end of the α_1 and α_2-chains, is unlikely to have occurred by chance. Its significance may lie in the fact that it represents a 40 Å length of extremely stable triple helix on the criteria postulated providing that the relative chain stagger is the minimum of one residue translation. All other values of *m* and *n* would lead to a predicted reduction in the stability of this portion of the molecule. As the formation of triple helix has been shown to commence in the vicinity of the C-terminal end of the molecule[16] the (Gly–imino–imino)$_5$ sequence may represent the initiating step in the formation of the triple helical collagen molecule.

DISCUSSION

Rauterberg and Kuhn[17] showed that modification of the charged side chains of collagen had a profound effect on fibril formation but appeared to be unimportant in the formation of triple helix. Hulmes *et al.*[2] when calculating the number of intermolecular interactions, showed that the polar interactions were about three times as numerous as the apolar interactions. This latter result agrees well with the conclusions of Rauterberg and Kuhn[17] providing that the weighting of the calculated polar and apolar interactions are similar. However, in the triple helix formation, this work has indicated that the apolar interactions are about three times as numerous as the polar interactions. Consequently, modification of the charged groups should have a much less significant effect on triple helix formation than on fibril formation, again in agreement with Rauterberg and Kuhn[17]. However, the level of significance of the polar interactions in these present calculations suggests that the polar interactions have both a critical and sensitive role in the axial arrangement of the α-chains with respect to one another. Therefore apolar interactions dominate formation of triple helix and polar interactions dominate fibril formation, though the contribution of the polar and apolar interactions respectively is clearly important.

In summary, it has been shown that whilst it is the homology between the α_1 and α_2-chains which leads to a one residue translation relative stagger between α-chains, it is probably the differences between the α_1 and α_2-chains particularly in the non-helical portions of the procollagen molecule which determine the ordering of the chains.

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