Proc. Natl. Acad. Sci. USA
Vol. 76, No. 10, pp. 5119–5123, October 1979
Biophysics

β-Turns in nascent procollagen are sites of posttranslational enzymatic hydroxylation of proline

[collagen/hydroxyproline/prolyl hydroxylase (proline,2-oxoglutarate dioxygenase)]

SAMIR K. BRAHMACHARI AND V. S. ANANTHANARAYANAN*

Molecular Biophysics Unit, Indian Institute of Science, Bangalore—560012, India

Communicated by Walter Kauzmann, June 13, 1979

ABSTRACT **The selective hydroxylation of proline residues in nascent procollagen chains by prolyl hydroxylase (EC 1.14.11.2) can be understood in terms of the conformational feature of the -Pro-Gly- segments in linear peptides and globular proteins. The folded β-turn conformation in such segments appears to be the conformational requirement for proline hydroxylation. The available data on the hydroxylation of native and synthetic substrates of prolyl hydroxylase are explained on the basis of the extent of β-turn formation in them. Taken in conjunction with the conformational features of the hydroxyproline residue, our results bring out the conformational reason for the posttranslational proline hydroxylation which, it is proposed, leads to the "straightening" of the β-turn segments into the linear triple-helical conformation.**

Hydroxyproline in collagen offers additional stability to the triple-helical conformation of this protein (1, 2). The hydroxylation of selected proline residues in the nascent chains of procollagen is catalyzed by prolyl hydroxylase (EC 1.14.11.2); the enzymatic hydroxylation as well as the triple-helix formation take place while the procollagen chains are still bound to polyribosome (2, 3). Studies on the natural and synthetic substrates of prolyl hydroxylase have demonstrated that only those proline residues that occur in position 3 of the typical collagen triplet, -Gly-R_2-R_3-, are hydroxylated and that not all the third-position proline residues get hydroxylated to the same extent (2, 4; references in ref. 4). An explanation for these observations in conformational terms is presented in this paper, based on the available experimental and theoretical results on peptides and polypeptides related to collagen. In addition, the conformational necessity for the posttranslational proline hydroxylation leading to the formation of a stable triple-helical conformation is also brought out.

Polytripeptide substrates of prolyl hydroxylase

As a prerequisite for the understanding of the substrate specificity of prolyl hydroxylase, the study of the conformation of polytripeptides of the type $(Gly\text{-}Pro\text{-}X)_n$ and $(Gly\text{-}X\text{-}Pro)_n$ has been undertaken in several laboratories, including our own (5–8). The results show that when X is Ala, Ser, Sar, or Leu, the $(Gly\text{-}Pro\text{-}X)_n$ polytripeptides adopt the triple-helical conformation more readily than the $(Gly\text{-}X\text{-}Pro)_n$ counterparts, which do not appear to possess any significant degree of periodically ordered conformation in aqueous solutions. The former type of polytripeptides were found not to interact with prolyl hydroxylase, whereas the latter acted as good substrates (9). The enzymatic hydroxylation of the proline residues in $(Gly\text{-}Pro\text{-}Pro)_n$ and in protocollagen (which is the unhydroxylated form of procollagen) is found to take place only when the reaction temperature is higher than the respective melting temperatures of the polymers, below which they exist in the triple-helical conformation (2). These data, in conjunction with the fact that native collagen is a poor substrate for the enzyme, have led to the conclusion that prolyl hydroxylase cannot act on substrates that already possess the triple-helical conformation (2, 9). When the substrate molecule exists in the disordered state, the enzyme appears to require the presence of the Pro-Gly bond, as seen from its action on $(Gly\text{-}X\text{-}Pro)_n$ and $(Gly\text{-}Pro\text{-}Pro)_n$. In addition, as pointed out by Prockop *et al.* (2), prolyl hydroxylase "reads" the peptide substrates in the order -X-Pro-Gly-. This is illustrated by the fact that the simple tripeptide Gly-Pro-Pro is not a substrate but Pro-Pro-Gly is. With a view to understanding the conformational basis for the specificity of prolyl hydroxylase, we have examined the conformation of Pro-Gly-containing segments in peptides and proteins.

Conformational features of -Gly-Pro- and -Pro-Gly- segments

From theoretical considerations (10, 11), the basic difference between the conformation of the peptides containing either the -Pro-Gly- or -Gly-Pro- segments is that the -Gly-Pro- sequence energetically favors an "extended" conformation (with $\phi_{Gly} = 178°$, $\psi_{Gly} = 175°$, $\phi_{Pro} = -75°$, and $\psi_{Pro} = 79°$), as shown in Fig. 1, and can readily adopt a polypeptide II type conformation (12). On the other hand, the -Pro-Gly- sequence favors a folded conformation similar to the type II β-turn (13) as one of the low-energy conformations (Fig. 1).

It is thus clear that the placement of the Pro residue (rather than the X residue) with respect to Gly in a given tripeptide sequence is what dictates the conformation of this segment. The presence of -Pro-Gly- sequences in the polytripeptides of the type $(Gly\text{-}X\text{-}Pro)_n$, referred to in the earlier section, would tend to make the polypeptide chain take up a folded conformation. On the other hand, the presence of the -Gly-Pro- sequences in the polytripeptides of the type $(Gly\text{-}Pro\text{-}X)_n$ would allow the backbone to take up an extended (similar to polyproline II type) conformation, making them readily amenable to form the collagen-like triple-helical structure. This explains the observed conformational difference between these two types of polytripeptides, which was hitherto not clearly understood.

In the light of the above observations, we would like to propose that the conformational requirement for the enzymatic hydroxylation of the proline residues is the presence of a folded conformation, similar to the β-turn, in the substrate molecules. The enzyme would not act on molecules that possess the collagen-like triple-helical or polyproline II type conformation [as found in $(Gly\text{-}Pro\text{-}X)_n$ polymers]. Our experimental data on -Pro-Gly-containing peptides and our analysis of such peptide segments in globular proteins support the theoretically expected β-turn conformation in them, as described below.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

* To whom all correspondence should be addressed at the Department of Biochemistry, Memorial University of Newfoundland, St. John's, Newfoundland A1B 3X9, Canada.

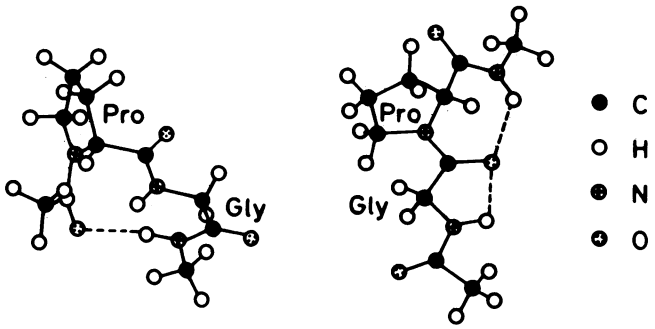

FIG. 1. Minimum energy conformation of Ac-Pro-Gly-$NHCH_3$ (*Left*) and Ac-Gly-Pro-$NHCH_3$ (*Right*) with all *trans* peptide unit (redrawn from ref. 10).

Studies on peptides

Spectroscopic studies (14, †) using circular dichroism NMR, infrared and, where possible, x-ray techniques on Ac-Pro-Gly-X-OH, in which X = Gly, Ala, Leu, Ile, and Phe, clearly point to the presence of the β-turn conformation in these tripeptides.‡ In addition, they reveal another interesting aspect, namely, the influence of the nature of the residue X on the extent of β-turn formation by these peptides in solution. In the order of decreasing effectiveness in stabilizing the β-turn conformation, the finding is that Leu > Ala > Gly, Ile > Phe. There is thus an indication that the extent of the enzymatic hydroxylation of proline residues, which is known (2, 9) to be influenced by the nature of the adjoining residue (X in the -Pro-Gly-X- repeating sequence), may be related to the extent of stabilization of the β-turn by this residue in given tripeptide segment (see later).

Studies on proteins

Chou and Fasman (16) have recently made a statistical analysis of the conformational preferences of the amino acid residues in the four possible positions of the β-turn, using the crystal structure data on 29 globular proteins. This analysis showed that Pro in the second and Gly in the third position of the β-turn have relatively very high β-turn potentials. This is in conformity with the experimental data on peptides (10, 14, 17, †).

Additional evidence for the preference of Pro-Gly segments to adopt the β-turn and for the influence of the neighboring X residue on β-turn formation is obtained from our analysis (14, §) of the conformation of tetrapeptide segments of the type Z-Pro-Y-X and Z-Pro-Gly-X in 33 globular proteins of known three-dimensional structure (Z, Y, and X represent amino acid residues). These studies place the amino acid residues broadly in three groups depending on their stabilizing, destabilizing, or neutral effect on the formation of the β-turn when these residues are present in position X of the above tetrapeptide segments. A good correlation is found between these data and the experimental studies on the tripeptides mentioned earlier with regard to the stability of the β-turn formation as governed by the nature of the residue at the COOH-terminal side of the Pro-Gly segment.

† S. K. Brahmachari, V. S. Ananthanarayanan, R. S. Rapaka, and R. S. Bhatnagar, unpublished.

‡ The vacuum-UV circular dichroism of the Leu tripeptide in β-turn conformation has been published (15).

§ Brahmachari, S. K. & Ananthanarayanan, V. S. (1978) Paper presented at the International Symposium on Biomolecular Structure, Conformation, Function and Evolution, Madras, India.

Additional evidence for β-turn as the conformational requirement for prolyl hydroxylase action

That the conformational requirement for prolyl hydroxylase action is the presence of β-turn in the substrate molecule at the Pro-Gly segment is substantiated not only by the data on native and denatured collagen, polyproline, and the model polytripeptides referred to earlier but also by those on several other systems. As illustrations, the following observations may be cited.

(*i*) Prockop *et al.* (2) found from model tripeptide studies that the prolyl hydroxylase "reads" the peptide as X-Pro-Gly rather than Gly-Pro-X. These authors also observed that Pro-Pro-Gly-$NHCH_3$ gets hydroxylated with the same order of magnitude of the turnover number as that of the ideal substrate, protocollagen. The major conformation of this tripeptide in solution is very likely to be the β-turn, as found by Stimson *et al.* (10) in the analogous Ac-Pro-Gly-$NHCH_3$. This would also indicate that the conformational specificity of prolyl hydroxylase lies at the level of the basic tripeptide unit in natural substrates.

(*ii*) In bradykinin, which has three proline residues in its nine-residue-long chain, only the proline residue at position 3 gets hydroxylated by prolyl hydroxylase (18). The sequence around this proline residue is -Pro^2-Pro^3-Gly^4-Phe^5-. In an analogous compound, Ac-Pro-Gly-Phe-OH, we have demonstrated the presence of the type II β-turn by single-crystal x-ray crystallography (14).

(*iii*) A recent report by Bhatnagar *et al.* (19) on the enzymatic hydroxylation of several -Pro-Gly-containing synthetic polypeptide analogs of elastin reveals significant amounts of hydroxylation of proline in these polypeptides, and these polypeptides have been shown by spectroscopic techniques to possess a poly β-turn conformation (17).

(*iv*) $(\beta\text{-Ala-Pro-Pro})_n$ of relatively short chain length, which does not possess the triple-helical conformation in aqueous solution at 37°C (14, †) and can be expected to have β-turn type of folded conformation at the -Pro-β-Ala- segments, undergoes significant hydroxylation (9). Similarly, $(\text{Gly-Pro-Pro})_n$ undergoes hydroxylation only when the molecule is in the "disordered" conformation (above 37°C) and not when it takes up the triple-helical conformation (at lower temperatures) (2). It is likely that, at the higher temperatures, this polytripeptide contains several segments with the β-turn conformation.

Extent of hydroxylation

Both in synthetic polytripeptides of the type $(\text{Pro-Gly-X})_n$ and in native collagen the experimental observation has been that the extent of hydroxylation of the proline residue varies depending on the sequence (2, 9). As a corollary to our finding on the conformational requirement for proline hydroxylation, we might expect the extent of hydroxylation to be a function of the probability that a given sequence adopts the β-turn conformation.

Our studies on Ac-Pro-Gly-X-OH tripeptides have shown that Leu and Ala in the position X favor the β-turn, whereas residues with the side chain branched at the β carbon atom do not favor β-turn formation (14, †). The hydroxylation studies by Bhatnagar and Rapaka (9) have shown that the polymer $(\text{Pro-Gly-X})_n$ undergoes significant (10–30%) hydroxylation when X is Pro, Leu, or Ala but does not show any significant interaction with the polymer if X is Val. As seen earlier, our protein data analysis also showed that Val at the fourth position (X) of the Z-Pro-Gly-X sequence does not favor the β-turn conformation. This provides an answer why Leu and Val, both having bulky side chains, interact differently with prolyl hydroxylase.

Table 1. p_t values and extent of proline hydroxylation in the $\alpha 1$ and $\alpha 2$ chains of collagen*

Peptide fragment	Tetrapeptide sequence	p_t† × 10^4	Local polyproline II conformation‡
α1-CB2 30–33	Pro-Hyp-Gly-Ala	5.6	
42–45	Pro-Hyp§-Gly-Glu	7.2	+
45–48	Glu-Hyp-Gly-Glu	4.1	+
48–51	Glu-Hyp-Gly-Ala	3.1	
α1-CB4 60–63	Pro-Hyp¶-Gly-Pro	6.3	+
63–66	Pro-Hyp¶-Gly-Lys	8.4	+
75–78	Lys-Pro‖-Gly-Arg	2.6	
78–81	Arg-Hyp¶-Gly-Gln	5.4	
α1-CB7 594–597	Ala-Pro‖-Gly-Lys	4.0	
657–660	Gln-Hyp‖-Gly-Ala	3.4	
771–774	Ala-Hyp‖-Gly-Ala	2.6	
783–785	Thr-Pro‖-Gly-Pro	4.5	+
α2-CB4 27–30	Pro-Hyp-Gly-Ala	5.6	
42–45	Pro-Hyp-Gly-Glu	7.3	
48–51	Glu-Hyp-Gly-Gln	5.6	
63–67	Pro-Hyp¶-Gly-Lys	8.4	+
75–78	Lys-Pro‖-Gly-Arg	2.6	
84–87	Val-Pro‖-Gly-Pro	2.9	
96–99	Thr-Hyp¶-Gly-Leu	4.7	
99–102	Leu-Hyp¶-Gly-Phe	3.4	
117–120	Gln-Hyp§-Gly-Ala	3.4	
180–183	Pro-Hyp¶-Gly-Phe	7.0	+
183–186	Phe-Hyp¶-Gly-Ala	3.5	+
186–189	Ala-Hyp¶-Gly-Pro	3.0	+
213–216	Leu-Hyp¶-Gly-Leu	3.3	
222–225	Pro-Hyp¶-Gly-Asn	8.3	+
288–291	Glu-Hyp¶-Gly-Ala	3.1	+
294–297	Gln-Hyp¶-Gly-Pro	3.9	+
297–300	Pro-Hyp¶-Gly-Pro	6.3	+
318–321	Pro-Hyp¶-Gly-Pro	6.3	+
321–324	Pro-Hyp¶-Gly-Leu	6.7	+
333–336	Leu-Hyp¶-Gly-Ala	2.7	

* The sequence and hydroxylation data are from ref. 4 and original papers cited therein. Residue numbers correspond to those in collagen.
† Calculated by using the parameters given in ref. 16.
‡ As judged from relatively large proline content or successive occurrence of Gly-Pro-X (see text).
§ Under-hydroxylated (about 50%).
¶ Estimated 70–100% hydroxylated, not accurately determined.
‖ Almost unhydroxylated (about 10% hydroxylated).

It is, however, difficult to obtain a one-to-one correlation between the nature of the X-residue side chain and the extent of hydroxylation for all the residues, because the accuracy of the hydroxylation data (as to the degree of hydroxylation) and that of our own data (as to the extent of β-turn formation) are somewhat limited.

In order to support the general validity of this correlation, we have adopted a somewhat different approach (20), wherein the secondary structure of the collagen molecule was generated by using the method of Chou and Fasman (21) on the available sequence data on the $\alpha 1$ and $\alpha 2$ chains (4, 22). All the hydroxyproline residues were treated as proline residues in this analysis. We found that the molecule did not contain any α-helical or β-structure fragments but did possess a rather large number of segments with the β-turn conformation. The average β-turn probability $\langle p_t \rangle$ of a tetrapeptide sequence of the type R_2-Pro-Gly-R_2' will be about 6×10^{-4}, assuming R_2 and R_2' to have average $\langle f_1 \rangle$ and $\langle f_4 \rangle$ values, respectively, of 0.096 (see ref. 16; note also that this is higher than the $\langle p_t \rangle$ of a general tetrapeptide sequence, namely about 1×10^{-4} considered by Chou and Fasman). Computation of the p_t values for individual tetrapeptide sequences along the collagen polypeptide chain revealed that whereas the -Pro-Gly- segments were found mainly in the β-turn conformation, almost none of the -Gly-Pro- segments preferred this conformation.

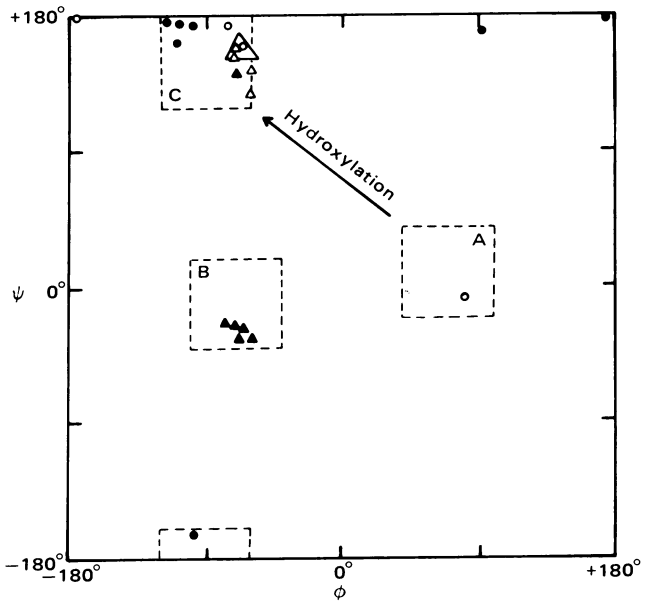

FIG. 2. ϕ, ψ values for -Gly-Pro- and -Pro-Gly- sequences in linear oligopeptides as obtained from crystal structure data (23). ○ and ●, the ϕ, ψ values of Gly in -Pro-Gly- and -Gly-Pro- sequences, respectively; △ and ▲, the ϕ, ψ values of Pro in the respective sequences. Region A indicated by broken lines corresponds to low ψ_{Gly} values found in type II β-turns. Region B corresponds to low ψ_{Pro} values; region C represents high ψ_{Gly} and high ψ_{Pro} values. The ϕ, ψ values of Pro and Gly in collagen and polyproline II fall in region C; the latter conformation is indicated by the large triangle in C.

Table 1 lists the p_t values for a few sets of data. It is to be noted that the prediction method used tends to overestimate the β-turn probability when there are a large number of Pro residues appearing in successive tetrapeptide sequences (21). We have, therefore, considered sequences of 8 residues at a stretch and assigned a polyproline II conformation to those that contain more than 50% Pro, even when the computed p_t values were higher than $\langle p_t \rangle$. (An example of such a "local" polyproline II conformation is found in the residue 58–63 sequence of cytochrome c_{551}.¶) It is interesting to note that there is a significant variation in the p_t values of different tetrapeptide sequences along the same polypeptide chain, these lying on either side of $\langle p_t \rangle$ (Table 1). This clearly indicates that not all the -Pro-Gly- segments in the nascent procollagen molecule exhibit the same preference for the β-turn conformation, this being governed by the nature of the adjoining residues, as was earlier seen from the tripeptide data. There is a fair, though not complete, correlation between the p_t value of a given segment and the extent of proline hydroxylation when the possible presence of the local polyproline II conformation is also taken into account. The correlation is better in cases in which hydroxylation is absent or incomplete. In particular, the absence of hydroxylation of residues 76, 595, and 784 of the $\alpha 1$ chain and of 76 and 85 of the $\alpha 2$ chain is accounted for by the relatively lower p_t values of the corresponding sequences.

¶ Dickerson, R. E., Almassoy, R. J. and Takano, T. (1978), paper presented at the International Symposium on Biomolecular Structure, Conformation, Function and Evolution, Madras, India.

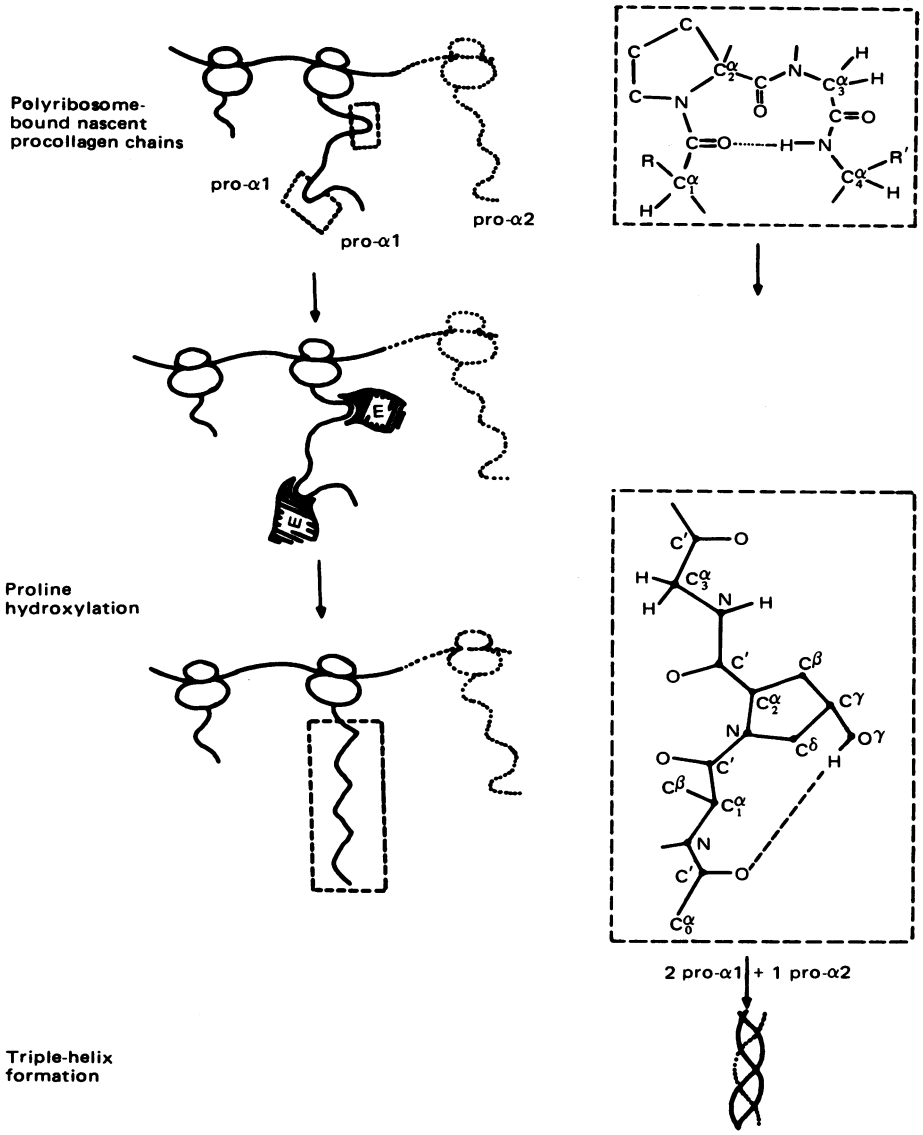

FIG. 3. Conformational events taking place during the proline hydroxylation in collagen biosynthesis. (The NH_2-terminal globular extensions on the nascent chains are not shown in the figure.)

Crystal structure data on linear peptides

It is of interest, at this stage, to investigate what conformations are likely for a -Pro-Gly- sequence, *other than* the type II β-turn. This exercise is useful, as will be shown in the following section, for understanding the conformational necessity for proline hydroxylation in collagen. For this purpose, we have looked into the available crystal structure data (23) on *t*-Boc-Pro-Gly-OH, *N*-pivalyl-Pro-Gly-OH, and Leu-Pro-Gly-OH in terms of their ϕ_{Pro}, ψ_{Pro}, ϕ_{Gly}, and ψ_{Gly} values, along with those of the tripeptide (Ac-Pro-Gly-Phe-OH), which we have found adopts the β-turn conformation in the crystal (unpublished data). These are plotted on the (ϕ, ψ) map in Fig. 2. It is extremely interesting to find that all three -Pro-Gly- that do not take up a β-turn conformation have their ϕ, ψ values clustered around the minimum energy conformation of polyproline II (region C in Fig. 2), in spite of the flexibility around the N—C^α and C^α—C′ bonds of the Gly residue.

We have also plotted in Fig. 2 the ϕ_{Gly}, ψ_{Gly}, ϕ_{Pro}, and ψ_{Pro} values for several of the available linear peptides with -Gly-Pro- sequences, namely, *t*-Boc-Gly-Pro-OH, *t*-Boc-Gly-Pro-OBzl, Cbz-Gly-Pro-OH, Cbz-Gly-Pro-Leu-OH, *p*-Br-Cbz-Gly-Pro-Leu-Gly-OH, and *o*-Br-Cbz-Gly-Pro-Leu-Gly-Pro-OH (23). The interesting observation is that some of the Pro residues take up a low ψ value ($\approx -30°$) whereas others fall in the $\psi = 150°$ region. On the other hand, the Gly residues in all these peptides have relatively high ψ values ($\approx 170°$), which are close to the value found for Gly in collagen or in the polyproline II conformation.

The -Gly-Pro- and -Pro-Gly- sequences that are not in the β-turn conformation (i.e., region B in Fig. 2) will have to cross a barrier of about 1–2 kcal/mol (24) (1 kcal = 4.18 kJ) to attain the ϕ, ψ required to form the triple-helical or polyproline II structure (region C in Fig. 2). In contrast, the -Pro-Gly- sequences that adopt the type II β-turn conformation ($\phi_{Gly} \approx 80°$ and $\psi_{Gly} \approx 0°$, region A in Fig. 2) have to cross a higher energy barrier of at least 4–5 kcal/mol (24) to go over to the ϕ, ψ region of the triple-helical or polyproline II conformation.

Need for hydroxylation

The understanding of the conformational requirement of prolyl hydroxylase leads us to the following interesting observation on the need for the hydroxylation of proline residues in the nascent (i.e., unhydroxylated) procollagen molecule in terms

of the role of hydroxyproline residues in stabilizing the collagen structure (25). As the nascent procollagen molecule is being synthesized on the polyribosome, prolyl hydroxylase recognizes the region of Pro-Gly segments that take up the β-turn conformation and hydroxylates the proline residues in them (to different extents, depending on the nature of the adjoining residues). Recent studies on the conformation of polyhydroxyproline (26, 27) as well as the NMR data of Torchia on $(Hyp\text{-}Gly)_n$ (28) clearly point out that the γ-hydroxyl group of the ith hydroxyproline residue would be involved in an *intra*molecular hydrogen bonding with the carbonyl oxygen of the $(i\text{-}2)$th residue in the polypeptide chain. In the present context, this would mean that the enzymatic conversion of a Pro-Gly segment in nascent procollagen into Hyp-Gly would lead to the "straightening out" of the original "folded" β-turn conformation into a "rigid" conformation, as shown schematically in Fig. 3. Such a process is equivalent to the translation of the Pro-Gly segment from its type II β-turn region A in Fig. 2 to the polyproline-II region C, as indicated by the arrow in the figure. The energy requirement for this process can be met from the formation of the *intra*chain hydrogen bonding. The straightening of the individual polypeptide chains of procollagen in this way by the enzymatic conversion of specific proline residues to hydroxyproline becomes a conformational necessity for the alignment of three such chains and their supercoiling into the triple-helical collagen structure [wherein the hydroxyproline residues in a given chain can enter into *inter*chain hydrogen bonding (29)].

An implication of the hypothesis presented above is that polytripeptides of the type $(Gly\text{-}X\text{-}Hyp)_n$ would be triple-helical in conformation, in contrast to their proline counterparts $(Gly\text{-}X\text{-}Pro)_n$. It is gratifying to note that Rao and Adams (30) have independently demonstrated the collagen-like conformation of $(Ala\text{-}Hyp\text{-}Gly)_n$, whose unhydroxylated counterpart is known to be unordered (5, 6).

Recently, Rapaka *et al.* (31) have attempted to understand the conformational specificity of prolyl hydroxylase by performing conformational energy calculations on X-Pro-Gly sequences. They found that the interactions between X and Pro as reflected in their respective dihedral angles ψ_1 and ψ_2 played a role in the enzymatic hydroxylation. No consideration, however, was given to the conformation of the Pro-Gly segment, which, as we have shown in this paper, is the key factor for the hydroxylation.

Finally, it is well known that in globular proteins, the β-turns occur at the exterior regions and are thus exposed to the action of solvents and other agents, such as enzymes. Our finding that the presence of the β-turn conformation is a prerequisite for posttranslational enzymatic hydroxylation of proline residues in collagen is thus reasonable from the point of view of the accessibility of the β-turn Pro-Gly segments to the enzyme. We are tempted to believe that the requirement of β-turn (or similarly folded) segments in protein substrates for posttranslational enzymatic action could be a very general one. In support of this, we may cite the observations that the sites of attachment of the sugar residues in glycoproteins (32, 33) and the sites of phosphorylation (34) are those that adopt the β-turn conformation. Preliminary examination of the site of hydroxylation of lysine residues in collagen and that of methylation of histidine in G-actin appears to support this generalization.

This work was supported by a grant from the Department of Science and Technology (India).

1. Ramakrishnan, C. & Ramachandran, G. N. (1976) in *Biochemistry of Collagen*, eds. Ramachandran, G. N. & Reddi, A. H. (Plenum, New York), pp. 45–84.
2. Prockop, D. J., Berg, R. A., Kivirikko, I. & Uitto, J. (1976) in *Biochemistry of Collagen*, eds. Ramachandran, G. N. & Reddi, A. H. (Plenum, New York), pp. 163–273.
3. Veis, A. & Brownwell, A. G. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 902–905.
4. Peiz, K. A. (1976) in *Biochemistry of Collagen*, eds. Ramachandran, G. N. & Reddi, A. H. (Plenum, New York), pp. 1–44.
5. Doyle, B. B., Traub, W., Lorenzi, G. P. & Blout, E. R. (1971) *Biochemistry* **10,** 3052–3060.
6. Brown, F. R., Di Corato, A., Lorenzi, G. P. & Blout, E. R. (1972) *J. Mol. Biol.* **63,** 85–99.
7. Scatturin, A., Tamburro, A. M., Del Pra, A. & Bordignon, E. (1975) *Int. J. Pept. Protein Res.* **7,** 425–435.
8. Ananthanarayanan, V. S., Brahmachari, S. K., Rapaka, R. S. & Bhatnagar, R. S. (1976) *Biopolymers* **15,** 707–716.
9. Bhatnagar, R. S. & Rapaka, R. S. (1976) in *Biochemistry of Collagen*, eds. Ramachandran, G. N. & Reddi, A. H. (Plenum, New York), pp. 479–523.
10. Stimson, E. R., Zimmerman, S. S. & Scheraga, H. A. (1977) *Macromolecules* **10,** 1049–1060.
11. Zimmerman, S. S. & Scheraga, H. A. (1977) *Biopolymers* **16,** 811–843.
12. Sasisekharan, V. (1959) *Acta Crystallogr.* **12,** 897–903.
13. Venkatachalam, C. M. (1968) *Biopolymers* **6,** 1425–1436.
14. Brahmachari, S. K. (1978) Dissertation (Indian Institute of Science, Bangalore, India).
15. Brahmachari, S. K., Ananthanarayanan, V. S., Brahms, J., Brahms, S., Rapaka, R. S. & Bhatnagar, R. S. (1978) *Biochem. Biophys. Res. Commun.* **86,** 605–607.
16. Chou, P. Y. & Fasman, G. D. (1977) *J. Mol. Biol.* **115,** 135–175.
17. Urry, D. W., Ohnishi, T., Long, M. & Mitchell, L. W. (1975) *Int. J. Pept. Protein Res.* **7,** 367–378.
18. Cardinale, G. J. & Udenfriend, S. (1974) *Adv. Enzymol. Relat. Area Mol. Biol.* **41,** 245–300.
19. Bhatnagar, R. S., von Dohlen, F., Sorenson, K. R., Rapaka, R. S. & Urry, D. W. (1978) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **37,** 1527.
20. Brahmachari, S. K. & Ananthanarayanan, V. S. (1978) *Curr. Sci.* **47,** 107–108.
21. Chou, P. Y. & Fasman, G. D. (1974) *Biochemistry* **13,** 222–245.
22. Fietzek, P. P. & Rexrodt, F. W. (1975) *Eur. J. Biochem.* **59,** 113–118.
23. Benedetti, E. (1977) *Peptides*, Proceedings of the Fifth American Peptide Symposium, eds. Goodman, M. & Meienhofer, J. (Wiley, New York), p. 257–273.
24. Kolaskar, A. S., Sarathy, K. P. & Sasisekharan, V. (1975) *Curr. Sci.* **44,** 35–38.
25. Berg, R. A. & Prockop, D. J. (1973) *Biochem. Biophys. Res. Commun.* **52,** 115–120.
26. Bansal, M., Brahmachari, S. K. & Sasisekharan, V. (1979) *Macromolecules* **12,** 19–23.
27. Brahmachari, S. K., Bansal, M., Ananthanarayanan, V. S. & Sasisekharan V. (1979) *Macromolecules* **12,** 23–32.
28. Torchia, D. A. (1972) *Macromolecules* **5,** 566–569.
29. Bansal, M., Ramakrishnan, C. & Ramachandran, G. N. (1975) *Proc. Indian Acad. Sci.* **82A,** 152–164.
30. Rao, N. V. & Adams, E. (1979) *Biochem. Biophys. Res. Commun.* **86,** 654–660.
31. Rapaka, R. S., Renugopalakrishnan, V., Urry, D. W. & Bhatnagar, R. S. (1978) *Biochemistry* **17,** 2892–2898.
32. Nagaraj, M. & Rao, V. S. R. (1977) *Curr. Sci.* **46,** 395–400.
33. Beely, J. G. (1977) *Biochem. Biophys. Res. Commun.* **76,** 1051–1055.
34. Small, D., Chou, P. Y. & Fasman, G. D. (1977) *Biochem. Biophys. Res. Commun.* **79,** 341–346.