CONFORMATIONAL CRITERIA FOR THE ENZYMATIC HYDROXYLATION OF PROLINE IN COLLAGEN

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ABSTRACT

With a view to understanding the conformational specificity of peptidyl proline hydroxylase, we have generated the secondary structure of the unhydroxylated collagen chain based on the available amino acid sequence data, using the procedure of Chou and Fasman. It is found that the probability of occurrence of a given chain segment, –Z–Pro–Gly–X– (where Z and X are amino acid residues) in the β-turn conformation, has a good correlation with the degree of hydroxylation of the proline residue in the segment by the enzyme. The enzyme, therefore, seems to recognize the β-turn as the conformational feature in the substrate. The hydroxylation of the proline residue results in the straightening of the β-turn regions, giving rise to a rigid chain. The available data on the hydroxylation of natural and synthetic substrates by the enzyme, are explained in a unified fashion by our hypothesis.

WE propose here a hypothesis on the conformational specificity of the enzyme, peptidyl proline hydroxylase (PPH) (EC 1.14.11.2), which hydroxylates the proline residues in the nascent procollagen molecule. In the absence of a specific codon for hydroxyproline, the acquisition of this imino acid residue, by collagen, is achieved by the hydroxylation of some of its proline residues by PPH. It is known[1] that (*a*) only those proline residues which are present in the 3rd position in the repeating sequence, –Gly–R_2–R_3– of the collagen polypeptide chain, get hydroxylated by PPH and (*b*) not all the proline residues in the 3rd position get hydroxylated to the same extent (R_2 and R_3 can be either an amino or imino acid in collagen). This is also true with synthetic polytripeptide models of the types $(Gly–X–Pro)_n$ and $(Gly–Pro–X)_n$ (where X is an amino acid). In order to understand the conformational reasons for this highly specific action of PPH, we have made a detailed analysis of the solution conformation of some of the polytripeptide models. These include[2,3] $(Gly–Sar–Pro)_n$ and $(Gly–Pro–Sar)_n$, $(Gly–Phe–Pro)_n$. In brief, our results, as well as those of others on other model polytripeptides[1], indicate that while $(Gly–X–Pro)_n$ is generally devoid of any periodic ordered structure, $(Gly–Pro–X)_n$ takes up a collagen-like triple-helical conformation in water. Taken in conjunction with the fact[1] that the triple-helical polytripeptides [*i.e.*, $(Gly–Pro–X)_n$] as well as native collagen and polyproline are poor substrates for PPH, the structural studies indicated the conformational flexibility in the substrate molecule, perhaps around the Pro–Gly bond, as a requirement for the hydroxylation. This, however, does not provide an answer, either to the exact nature of the conformational feature of the substrate molecule responsible for hydroxylation or to the varying extents of hydroxylation.

In order to understand the exact nature of the conformational feature of the Pro–Gly regions, we have carried out the following analysis. Treating all the hydroxyproline as proline residues in the available amino acid sequence data, on the α–1 and α–2 chains of collagen[4,5], we have generated the secondary structure for those chains using the method of Chou and Fasman[6]. We find that the molecule does not contain any α-helical or β-structure fragments but does contain a rather large number of segments with the β-turn conformation[7]. Computation of the probability, p_t, for individual tetrapeptide sequences, along the polypeptide chain revealed that while the Pro–Gly sequences were mainly found in the β-turn conformation, not all such sequences adopted this conformation. (These results are in conformity with our recent findings[8] based on X-ray crystal structure data on globular proteins, which show that the nature of the side chain of the fourth residue, X, in the sequence –Z–Pro–Gly–X, influences the β-turn propensity of this segment. Our experimental data[9] on tripeptide model compounds, forming the β-turn, also support these results.) We then made the interesting observation that there is a very good correlation between the magnitude of the p_t value of a given tetrapeptide sequence in collagen and the extent of hydroxylation of proline in that sequence

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by PPH. A typical set of the rather large number of our data analysis is shown in Table I.

TABLE I

β-turn probability p_t *and enzymatic hydroxylation data on the α-1 (I) CB2 fragment (residues 20-55) of rat skin or tendon collagen*[a]

Sequence number of proline residue within the fragment	$p_t \times 10^1$ for Z-Pro-Gly-X sequence[b]	% hydroxylation by PPH
9	>1·0	100
12	0·92	100
15	>1·00	100
24	0·67	20–40
27	<0·1	10–20
30	0·24	10-20

[a] Hydroxylation data taken from reference 15.
[b] p_t was computed as described in reference 6.

It is thus clear that *the conformational requirement for PPH action is the presence of β-turn in the substrate molecule at the –Pro–Gly–segment* (The Gly–Pro–containing tetrapeptide sequence, corresponding to the second position proline in the repeating tripeptide sequence of collagen, does not favour a β-turn[10].) The extent of hydroxylation of the proline residue at the β-turn depends on its relative stability in this conformation. It is known from our recent theoretical and experimental analysis of the polyhydroxyproline conformation[11], and from other studies[12] that the formation of a hydrogen bond between the OγH of the Hyp and the backbone C=O group, renders the given chain segment conformationally rigid. We may thus conceive of the action of PPH and the purpose of hydroxylation of Pro as one of recognizing the β-turn (*i.e.*, "chain reversal") portions of the procollagen molecule, hydroxylating the proline residues at the bends to Hyp (to varying extents) rendering the chain stiff and linear and thereby making it amenable for aggregation with two other similar chains to form the final triple helical conformation of native collagen. It should be mentioned that this hypothesis offers a unified explanation for a large number of apparently uncorrelated data on proline hydroxylation by PPH, including the recent data of Berg *et al.*[13], and the data on bradykinin[14]. A detailed description of the interpretation of these data will be published elsewhere.

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