# INTERACTION OF POLYPEPTIDE MODELS OF ELASTIN WITH PROLYL HYDROXYLASE

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## 1. Introduction

Significant amounts of hydroxyproline occur in the structural proteins collagen and elastin which usually exist in close association and are presumably synthesized by the same cells. Hydroxyproline in collagen is synthesized by the hydroxylation of proline residues in –X–Pro–Gly– sequences, by prolyl hydroxylase (EC 1.14.11.2) in a concerted reaction in which an obligatory co-substrate, α-ketoglutarate is oxidatively decarboxylated (reviewed in [1]). Much of the proline in elastin occurs in –Pro–Gly– sequences and therefore is potentially hydroxylatable by prolyl hydroxylase. Both collagen and elastin contain repeating sequences. We have examined the interaction of synthetic polymeric models of elastin with prolyl hydroxylase. Our studies suggest that proline in elastin-like polymers is efficiently hydroxylated under conditions where these polymers exhibit preferred secondary and tertiary structures. These studies explain the presence of hydroxyproline in the non-polar polymeric regions of elastin [2].

## 2. Materials and methods

The syntheses of $(Val–Pro–Gly–Gly)_n$ [3]; $(Ala–Pro–Gly–Gly)_n$ [4]; $(Val–Ala–Pro–Gly)_n$ [5]; $(Val–Pro–Gly–Val–Gly)_n$ [6] and $(Val–Ala–Pro–Gly–Val–Gly)_n$ [7], have been described. All polymers used in these studies were comparable in their degree of polymerization ($n \simeq 40$). $(Pro–Pro–Gly)_n$ (mol. wt 2400) was obtained from Miles Laboratories and was used without fractionation as an internal control in the hydroxylase assay. Interaction of the polypeptides as substrates of prolyl hydroxylase or as inhibitors of hydroxylation of the natural substrate was examined as in [8].

## 3. Results

If a substrate analogue is present in an enzymatic reaction mixture, its interaction with the enzyme decreases the reaction of the natural substrate. The presence of several polypeptide analogues of elastin reduced the hydroxylation of [$^3$H]proline in unhydroxylated collagen, by chick embryo prolyl hydroxylase (table 1). Because the polymers were used in equimolar amounts (25 μM) these data reflect their relative efficiencies of interaction with prolyl hydroxylase. On this basis, the elastin-related polypentapeptide $(Val–Pro–Gly–Val–Gly)_n$ showed the strongest interaction with the enzyme. Considerably lower efficiency of inhibition was observed with two other polymers of elastin-derived sequences, $(Val–Pro–Gly–Gly)_n$ and $(Val–Ala–Pro–Gly–Val–Gly)_n$. Significant inhibition was also seen with $(Ala–Pro–Gly–Gly)_n$ and $(Val–Ala–Pro–Gly)_n$. This experiment also indicated that the same prolyl hydroxylase may interact with collagen or elastin polypeptides.

The experiments in table 1 were carried out at 37°C. Under these conditions the reaction mixtures containing the polypentapeptide and the polyhexapeptide exhibited a gel-like appearance. These polymers have been shown to exhibit a concentration-dependent aggregation at 37°C [9], and as such could

Table 1
Relative inhibition of hydroxylation of unhydroxylated collagen by synthetic polypeptides at 37°C

| Addition | $^3$HHO released (dpm × $10^{-5}$) | % Inhibition |
|---|---|---|
| Control | 6.24 | – |
| $(Val–Pro–Gly–Gly)_n$ | 5.29 | 15 |
| $(Val–Pro–Gly–Val–Gly)_n$ | 1.67 | 73 |
| $(Val–Ala–Pro–Gly–Val–Gly)_n$ | 5.05 | 19 |
| $(Val–Ala–Pro–Gly)_n$ | 5.30 | 15 |
| $(Ala–Pro–Gly–Gly)_n$ | 4.07 | 35 |

The reaction was carried out at 37°C as in [1,7]. Reaction mixtures containing $(Val–Pro–Gly–Val–Gly)_n$ or $(Val–Ala–Pro–Gly–Val–Gly)_n$ became gel-like before the enzyme (3000-fold purified chicken embryo prolyl hydroxylase) was added. Tritiated water was collected by vacuum distillation and radioactivity determined in a scintillation counter

be co-aggregating with collagen, therefore, the interaction of these polymers with prolyl hydroxylase was also examined at 30°C. At this temperature the solutions remained clear. As seen in table 2 the rate of the hydroxylation reaction was reduced to nearly 10% of the rate at 37°C, however, the levels of inhibition by $(Val–Pro–Gly–Val–Gly)_n$, $(Val–Ala–Pro–Gly–Val–Gly)_n$ and $(Ala–Pro–Gly–Gly)_n$ were not different from those observed at 37°C suggesting that co-aggregation with collagen was not a dominant factor.

The interaction of the synthetic polypeptides with prolyl hydroxylase was also examined in terms of the hydroxylation of proline in these polymers. As seen in table 3, significant amounts of hydroxyproline were synthesized. These data with the exception of the polypentapeptide results show that the relative efficiencies of hydroxylation of the elastin-model polypeptides follow the same order as their ability to inhibit the hydroxylation of proline in unhydroxylated collagen. The hydroxylation of polypeptides was examined at 37°C and as in the case of the inhibition studies discussed above, reaction mixtures containing $(Val–Pro–Gly–Val–Gly)_n$ or $(Val–Ala–Pro–Gly–Val–Gly)_n$ appeared gel-like. However, as seen in the inhibition experiment, aggregation did not appear to affect polypeptide enzyme interaction leading to hydroxyproline synthesis perhaps due to the concentration of elastin peptides in the equilibrium solution. Because the rate of hydroxyproline synthesis at 30°C is only 10% of the rate at 37°C, the hydroxylation of polypeptides at 30°C could not be accurately assessed. The similarity of interaction in solution and in the aggregated state of the elastin-like polymers (Val–Pro–

Table 2
Relative inhibition of hydroxylation of unhydroxylated collagen by synthetic polypeptides at 30°C

| Addition | $^3$HHO released (dpm × $10^{-4}$) | % Inhibition |
|---|---|---|
| Control | 6.88 | – |
| $(Val–Pro–Gly–Val–Gly)_n$ | 1.75 | 74 |
| $(Val–Ala–Pro–Gly–Val–Gly)_n$ | 5.86 | 15 |
| $(Ala–Pro–Gly–Gly)_n$ | 4.38 | 36 |

The reaction was carried out at 30°C. All other conditions were the same as in table 1. No coacervation occurred under these conditions

Table 3
Relative hydroxylation of proline in synthetic polypeptide models of elastin

| Polymer | Hydroxyproline synthesized | |
|---|---|---|
| | μg Hyp/mg polymer | Hyp/1000 Pro[a] |
| $(Val–Pro–Gly–Gly)_n$ | 1.2 | 4 |
| $(Val–Pro–Gly–Val–Gly)_n$ | 2.2 | 10 |
| $(Val–Ala–Pro–Gly–Val–Gly)_n$ | 1.8 | 9 |
| $(Ala–Pro–Gly–Gly)_n$ | 5.4 | 16 |
| $(Val–Ala–Pro–Gly)_n$ | 6.0 | 15 |
| $(Pro–Pro–Gly)_n$ | 4.9 | 13 |

[a] Calculated on the basis of the susceptible proline residues (–Pro–Gly–)

The hydroxylation reaction was carried out at 37°C as in [7]

$Gly–Val–Gly)_n$ and $(Val–Ala–Pro–Gly–Val–Gly)_n$ may derive from the fact that there are molecules in solution which are in equilibrium with the aggregated molecules.

## 4. Discussion

Hydroxyproline occurs in significant amounts in collagen and a few other extracellular proteins. Variable amounts of hydroxyproline are found in elastin. Hydroxyproline contributes to the conformational stability of the collagen triple helix [10,11] by participating in hydrogen bond formation [12,13]. The mechanism of synthesis of hydroxyproline in elastin has not been fully investigated and its structural role in elastin has not been determined. Collagen and elastin occur in close association and are presumably synthesized in the same cells. Since the hydroxylation of proline in elastin sequences is catalyzed in vitro by the same enzyme which hydroxylates proline in collagen, it may be speculated that its presence in elastin arises from its incidental interaction with the enzyme.

Interactions between the side chain of the residue X with Pro, in the sequence –X–Pro– play a major role in determining the structural contributions of this sequence [14] and the coacervation properties of elastin-like polymers are regulated by these interactions [5,15]. Our studies on the hydroxylation of Pro in collagen-like polymers $(X–Pro–Gly)_n$ showed that the nature of the side chain at X may regulate hydroxylation [8,16]. From conformational energy calculations it was proposed that in an –X–Pro–Gly– sequence hydroxylation occurred if conformations with $\psi_1 = 100 \pm 40°C$ ($C^{\alpha}_{X} - C'^{O}$) and $\psi_2 = 130 \pm 30°C$ ($C^{\alpha}_{Pro} - C'^{O}$) predominate [8]. This limited conformational range is influenced by X–Pro interactions. Thus, $(Ala–Pro–Gly)_n$ is a better substrate for prolyl hydroxylase than $(Val–Pro–Gly)_n$. Hydroxylation of Pro in elastin-like polymers appears to be subject to similar stereochemical constraints. Thus the polymers $(Ala–Pro–Gly–Gly)_n$, $(Val–Ala–Pro–Gly–Val–Gly)_n$ or $(Val–Ala–Pro–Gly)_n$ with Ala on the N-terminal of Pro, are better substrates than $(Val–Pro–Gly–Gly)_n$. The exception appears to be $(Val–Pro–Gly–Val–Gly)_n$ which is a good substrate. The elastin peptides were shown to have a β-turn and it may be expected that collagen peptides of the X–Pro–Gly type may have some of the β-turn torsion angles before undergoing hydroxylation and therefore, that the β-turn may serve as a recognition site. The optimal $\psi_1$ and $\psi_2$ values proposed earlier for these peptides are component angles of a β-turn [8]. Recently, evidence has been presented that the β-turn has a regulating influence on protein phosphorylation [17], as the β-turn may be the site recognized by the phosphorylating enzymes. It has been suggested that the β-turn may serve as a recognition site in prolyl hydroxylase substrates [18]. However, the β-turn is not an obligatory requirement for hydroxylation as $(Pro–Pro–Gly)_n$ is an optimal

synthetic substrate, even though it can not form a $\beta$-turn, and neither is the $\beta$-turn a preemptive requirement as $(Val–Pro–Gly–Gly)_n$ hydroxylates poorly even though it forms a quite stable $\beta$-turn.

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