HYDROXYLATION OF POLY(L-PROLYL-L-PROLYLGLYCYL) OF DEFINED MOLECULAR WEIGHTS BY PROTOCOLLAGEN PROLINE HYDROXYLASE

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1. Introduction

Hydroxyproline in collagen is synthesized by the hydroxylation of proline residues in protocollagen, a collagen precursor [1]. The hydroxylating enzyme is an oxygenase [2,3] and is purified from chick embryos [4]. The latter authors studied the hydroxylation of polypeptides of various molecular weights, which were synthesized by polymerization of a tripeptide, L-prolylglycyl-L-proline, and fractionated by gel filtration. The molecular weight of the polymerization products had a distribution range.

Recently, Sakakibara et al. (including one of the authors) synthesized sequential polypeptides with defined molecular weights by stepwise addition of t-amyloxycarbonyl-L-prolyl-L-prolylglycine on the Merrifield resin [5]. The products, (L-prolyl-L-prolylglycyl)$_n$ or (Pro-Pro-Gly)$_n$, with n values bigger than ten showed a temperature-dependent transition in optical rotation and apparent molecular weight. The phenomenon was not observed with the above non-defined polymers. The transition was ascribed to the conformational change between single chain and triple stranded structures, similar to those observed with collagen [6]. The present paper describes the enzymatic hydroxylation of the products.

2. Methods

(Pro-Pro-Gly)$_n$, n = 1–20, were incubated with protocollagen proline hydroxylase. The preparation of the enzyme, the reaction and the hydroxyproline assay were carried out as described by Kivirikko and Prockop [4].

3. Results

Hydroxyproline formation in the presence of (Pro-Pro-Gly)$_n$, n = 1, 3, 5, 10, 15 and 20, in a total volume of 4.0 ml was determined. The amount of the enzyme and the substrates are given in the legends.

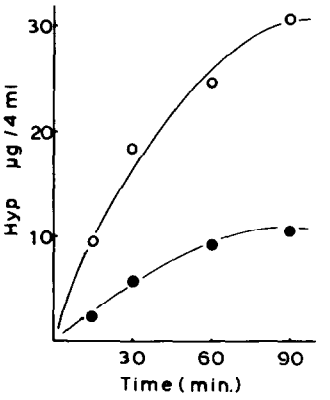

Fig. 1. Time course of the reaction. (Pro-Pro-Gly)$_3$ or (Pro-Pro-Gly)$_5$, 1 mg and enzyme protein, 7 mg in 4 ml of reaction mixture containing 0.04 mM $FeSO_4$, 0.5 mM α-ketoglutarate, 2 mM ascorbic acid and 0.05 M tris(hydroxymethyl)-aminomethane – HCl buffer, pH 7.8, was incubated at 37°. ●—● (Pro-Pro-Gly)$_3$; ○—○ (Pro-Pro-Gly)$_5$

Fig. 1 shows the time course of the reactions with a substrate, (Pro-Pro-Gly)$_3$ or (Pro-Pro-Gly)$_5$. Fig. 2

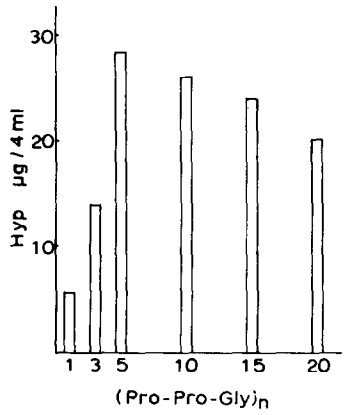

Fig. 2. Effect of chain length on the hydroxyproline formation. Each mg of $(Pro\text{-}Pro\text{-}Gly)_n$ and 7 mg of enzyme protein per 4 ml were incubated at 37° for 60 min in a similar reaction mixture to that in fig. 1.

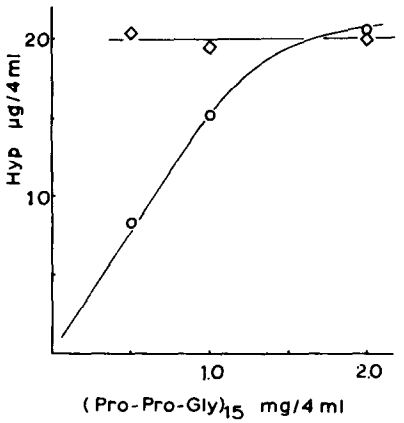

Fig. 3. Effect of pre-boiling of $(Pro\text{-}Pro\text{-}Gly)_{15}$ on the hydroxyproline formation. $(Pro\text{-}Pro\text{-}Gly)_{15}$ solution (2 mg per ml 0.2 M tris (hydroxymethyl)-aminomethane – HCl buffer, pH 7.8) was heated at 100° for 30 min just before the reaction. The peptide and enzyme protein, 10 mg, per 4.0 ml were incubated at 37° for 60 min in a similar reaction mixture to that of fig. 1. The unboiled control was also oxidized. ◇—◇ boiled $(Pro\text{-}Pro\text{-}Gly)_{15}$ ○—○ unboiled $(Pro\text{-}Pro\text{-}Gly)_{15}$.

shows the effect of chain length on the hydroxylation. There is a maximum at $n = 5$. The result is in contrast to that of Kivirikko and Prockop, who reported that the longer the peptide, the more hydroxylation was observed with preparations of molecular weight under 4000 [4].

The results are of interest considering that $(Pro\text{-}Pro\text{-}Gly)_{10}$ has a transition temperature at 25°, $(Pro\text{-}Pro\text{-}Gly)_{15}$ at 55° [7] and $(Pro\text{-}Pro\text{-}Gly)_{20}$ at 65° [5] and that the reaction was carried out at 37°.

The hydroxylation of $(Pro\text{-}Pro\text{-}Gly)_3$ or $(Pro\text{-}Pro\text{-}Gly)_5$, which does not form triple stranded conformation, was not affected by boiling the substrate solution at 100° for 30 min in advance of the reaction. With $(Pro\text{-}Pro\text{-}Gly)_{15}$, the hydroxylation was largely enhanced by the prior boiling as shown in fig. 3.

4. Discussion

A tripeptide, glycyl-L-prolyl-L-proline, is not hydroxylated by the enzyme [4] but the tripeptide, L-prolyl-L-prolylglycine, reported in this paper is hydroxylated.

With the peptides, $(Pro\text{-}Pro\text{-}Gly)_n$, of n values up to five, the longer the chain, the quicker was the hydroxylation. The amount of peptides in the reaction mixture was constant in weight. The molar concentration of the peptides, therefore, was inversely proportional to the n values. The results show that the increase in susceptibility to the enzyme with n exceeds the decrease in molar concentration.

The boiling of the peptide, $(Pro\text{-}Pro\text{-}Gly)_{15}$, is known to cause the destruction of the triple stranded conformation. The results shown in fig. 3 indicate that the "molten" state is more favourable to the enzymatic hydroxylation. On shorter peptides of n under five, which do not form triple stranded conformation, the boiling had no effect. There are two possibilities to explain the results: (A) the triple stranded substrate is not hydroxylated and only the monomer in equilibrium with the trimer or monomeric portion of a loose trimer is hydroxylated. (B) the apparent decrease in substrate concentration by trimer formation is the reason for the decrease in the hydroxylation. In either case, the triple stranded structure is unfavourable for the hydroxylation. The results agree with those of Fujimoto and Prockop that *Ascaris* cuticle collagen is more easily hydroxylated after denaturation [8], and disagree with those of some recent papers that boiled and unboiled protocollagen are hydroxylated equally [9–11].

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