Protocollagen Proline Hydroxylase from *Ascaris lumbricoides*  

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**SUMMARY**

An enzyme was found in the muscle layers of *Ascaris lumbricoides* which was similar to the protocollagen proline hydroxylase found in chick embryos and other vertebrates in that it synthesized $^{14}$C-hydroxyproline when incubated with $^{14}$C-proline-labeled protocollagen from chick cartilage. The *Ascaris* enzyme was also similar in that it required atmospheric oxygen, $\alpha$-ketoglutarate, iron, and ascorbate in order to synthesize $^{14}$C-hydroxyproline. A further similarity between the *Ascaris* hydroxylase and the one in chick embryos was that preparations of proline-labeled protocollagen from the cuticle of *Ascaris* served as substrates only after they were boiled.

The *Ascaris* hydroxylase differed from the chick embryo enzyme in that it was partially inhibited by incubation in normal atmospheric air, and the rate of the reaction increased by about 50% as the oxygen content of the gas phase was reduced to 1%. The $K_m$ for $\alpha$-ketoglutarate for the *Ascaris* enzyme was about $4 \times 10^{-4}$ M, or about 100-fold greater than the $K_m$ for $\alpha$-ketoglutarate with the chick embryo enzyme. A further difference was that the *Ascaris* enzyme was not inhibited by poly-L-proline II which competitively inhibits the chick embryo enzyme.

Two distinct types of collagen are present in *Ascaris lumbricoides*. The collagen isolated from the cuticle differs from collagen found in vertebrates in that it has a low content of hydroxyproline, a high content of proline, and essentially no hydroxylysine (1, 2). Also, physical measurements (2-4) and enzymatic studies (5) indicate that the molecule has an unusual conformation. The collagen found in the muscle layers of *Ascaris* has a higher content of hydroxyproline, hydroxylysine, and carbohydrate than most of the collagen in vertebrates (6).

Recent studies (for review, see Reference 7) have established that the hydroxyproline and hydroxylysine found in the collagen from vertebrates are synthesized by the hydroxylation of proline and lysine which have been incorporated into a large polypeptide precursor of collagen called protocollagen. A protocollagen proline hydroxylase purified from chick embryos does not hydroxylate free proline or proline in tripeptides, but it hydroxylates proline in biologically prepared protocollagen (7) or in polypeptides resembling collagen (8, 9). The protocollagen hydroxylase from chick embryos also hydroxylates proline in the collagen from the cuticle of *Ascaris lumbricoides* if the cuticle collagen is denatured, but native forms of the cuticle collagen have a conformation which prevents interaction with the enzyme (5). In work reported here an enzyme was prepared from the muscle layers of *Ascaris lumbricoides* which has a number of similarities to the protocollagen from chick embryos, but which differs from the chick embryo enzyme in its affinity for oxygen, $\alpha$-ketoglutarate, and poly-L-proline II.

**MATERIALS AND METHODS**

**Preparation of Protocollagen Hydroxylase from Chick Embryos—** Protocollagen proline hydroxylase was prepared from 12-day-old chick embryos with the procedures described previously (8). From 200 to 400 chick embryos were used, and the enzyme was purified through the calcium phosphate gel step. The enzyme was assayed with 125 µg per ml of the polytripeptide (Pro-Gly-Pro)$_n$ (8), and 1 unit of enzymatic activity was defined as the amount of enzyme required to synthesize 1 µg of hydroxyproline in 1 hour. As indicated previously (5, 8, 9), the specific activity of the enzyme preparations obtained by purification through the calcium phosphate gel step was more than 50 times the specific activity of crude extracts of chick embryos, and the various preparations used here had specific activities of 5 to 10 units per mg of enzyme protein.

**Preparation of Proline-labeled Protocollagen from Chick Embryos and from *Ascaris*—** The $^{14}$C-proline-labeled protocollagen from chick embryos was prepared by incubating cartilaginous tibiae from 10-day-old embryos with $^{14}$C-proline and 1 mM $\alpha,\alpha'$-dipyridyl (9). The soluble protein fraction from the tissue was dialyzed and boiled, and then it was frozen and stored in aliquots containing about 40,000 or 50,000 dpm each. As indicated
previously (9), each sample contained about 0.5 μg of proline-labeled protocollagen, and less than 100 dpm of 14C-hydroxyproline.

Ascaris lumbricoides (var. suis) were obtained from a local slaughterhouse in Philadelphia, Pennsylvania. The proline-labeled protocollagen from Ascaris was prepared by treating five worms with injection of 8 μC each of 14C-proline (New England Nuclear), 197 μC per μmole, and then incubating them in Locke's solution with 1 mM α,α'-dipyridyl at 37°C for 60 hours (10). The worms were frozen and thawed several times, and then they were cut longitudinally. The cuticles were detached, and were minced with scissors. The samples were ground in a mortar and pestle in the cold, and were then homogenized in 0.5 m NaCl in a Teflon and glass homogenizer. The protocollagen was extracted by shaking in the cold with 10 ml of 0.5 m NaCl for 48 hours. The sample was centrifuged at 15,000 × g for 20 min, and the supernatant fraction was dialyzed exhaustively against 0.05 m KCl and 0.02 m Tris-HCl buffer, pH 7.2. The samples were stored at 4°C. In order to prepare protocollagen from the muscle layers, the cuticles were detached from the worms, and the soft internal organs were removed by blunt dissection. The remainder of the worms was homogenized in 20 ml of water, and the homogenate was centrifuged at 15,000 × g for 20 min. The sediment was extracted with 10 ml of 0.5 m acetic acid for 3 hours at 4°C, and the sample was centrifuged at 15,000 × g for 20 min. The supernatant fraction was then dialyzed and stored under the same conditions as the cuticle protocollagen.

The preparation of cuticle protocollagen contained about 0.5 μg of hydroxyproline or about 20 μg of cuticle collagen per 70,000 dpm. Because the protocollagen preparations from Ascaris contained relatively large amounts of previously synthesized collagen, and because the rate of synthesis was relatively slow, their content of proline-labeled protocollagen could not be estimated in a manner similar to chick embryo protocollagen by measuring the net synthesis of hydroxyproline in the system with and without α,α'-dipyridyl (14). As indicated below, only about 10% of the 14C in the cuticle preparations was accounted for by 14C-hydroxyproline and 14C-proline in protocollagen.

**Conditions for Incubation of Enzyme**—The enzymatic reaction with the hydroxylase from chick embryos was carried out in a final volume of 8 ml which contained 0.23 unit of enzyme preparation; 50,000 dpm of 14C-proline-labeled protocollagen; 0.04 mM FeSO4; 0.5 mM α-ketoglutarate; 2 mM ascorbic acid (Fisher); 0.05 mg per ml of catalase (Calbiochem); and 50 mM Tris-HCl buffer adjusted to pH 7.8 at 25°C (8). The amount of enzyme was increased to 20 units in experiments designed to produce maximal hydroxylation of protocollagen isolated from either chick embryos or Ascaris. The enzymatic reaction with the enzyme from Ascaris was carried out under the same conditions except that the α-ketoglutarate concentration was increased to 1 mM.

After the sample had been incubated at 37°C with shaking for the times indicated, the reaction was stopped by the addition of 8 ml of concentrated HCl. The samples were hydrolyzed overnight in sealed tubes at 120°C, and the hydrolysates were evaporated to dryness in a rotary evaporator. The residues were dissolved in 4.0 ml of water, and the hydrolyzed samples were assayed for 14C-hydroxyproline with a specific chemical enzymatic assay even though the reaction was not linear for incubation periods greater than 15 min, probably because under the conditions used the substrate concentration is relatively low compared to the enzyme concentration (12). Similar results were obtained previously when proline-labeled protocollagen was used as a substrate for protocollagen hydroxylase from chick embryos (12) in that it was a soluble protein. Essentially all the enzymatic activity was recovered in the supernatant fraction obtained by centrifuging the homogenate at 100,000 × g for 1 hour.

Most of the enzymatic activity was in the muscle layers of the Ascaris, since the activity per mg of cuticle, wet weight, was only about 10% of the activity per mg of the muscle layer fraction.

**Hydroxylation of 14C-Proline-labeled Protocollagen from Chick Embryos**—The hydroxylation of proline-labeled protocollagen from chick embryos was found to be linear with time for about 15 min (Fig. 1). The rate of the reaction decreased with incubation periods greater than 15 min, probably because under the conditions used the substrate concentration is relatively low compared to the enzyme concentration (12). Similar results were obtained previously when proline-labeled protocollagen was used as a substrate for protocollagen hydroxylase from chick embryos (12). As with the chick embryo enzyme (12), it was found that 1-hour incubation periods could be used for enzymatic assays even though the reaction was not linear for this period of time. The amount of 14C-hydroxyproline synthesized in 1 hour was directly proportional to the amount of enzyme added (Fig. 2), and, in order to conserve both substrate...
Cofactors or cosubstrates required by protocollagen proline hydroxylase from *Ascaris*

$^{14}$C-Proline-labeled protocollagen (50,000 dpm) was incubated with the enzyme preparation obtained from the muscle layers of *Ascaris* under the conditions described in the text. In Experiment 1, 5.6 mg of one enzyme preparation was used, and in Experiment 2, 3.7 mg of another preparation was used. In Experiment 2, the samples were placed in Thunberg tubes, and after air was removed from one tube by evacuation with a water aspirator, the reaction was initiated by addition of the $\alpha$-ketoglutarate from the side arm.

<table>
<thead>
<tr>
<th>Incubation system</th>
<th>$^{14}$C-Hydroxyproline synthesized</th>
<th>Relative activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete system.</td>
<td>1730</td>
<td>100</td>
</tr>
<tr>
<td>$-\alpha$-ketoglutarate.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$-$ ascorbic acid.</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>$-Fe^{2+}$.</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>$-$ catalase.</td>
<td>1350</td>
<td>78</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete system.</td>
<td>1160</td>
<td>100</td>
</tr>
<tr>
<td>$-\alpha$-ketoglutarate.</td>
<td>68</td>
<td>1</td>
</tr>
</tbody>
</table>

and enzyme, 1-hour incubation periods were generally used for the assay.

The cofactors or cosubstrates required for the hydroxylation of chick embryo protocollagen by the *Ascaris* enzyme were the same as those required for the hydroxylation of chick embryo protocollagen by the chick embryo enzyme (7), and essentially no synthesis of $^{14}$C-hydroxyproline was observed when $\alpha$-ketoglutarate, ascorbate, ferrous iron, or oxygen was omitted from the system (Table I). Catalase, which was earlier found to improve the activity of some enzyme preparations from chick embryos (8), also helped to stabilize the *Ascaris* enzyme in some experiments. Addition of dithiothreitol (13) did not enhance the enzymatic activity of either the hydroxylase preparations from *Ascaris* or the purified hydroxylase from chick embryos (not shown).

Further investigation of the cofactor and cosubstrate requirements indicated two differences from the chick embryo hydroxylase. Although molecular oxygen was essential for the reaction (Table I), the normal atmospheric concentration of oxygen inhibited the reaction with the *Ascaris* enzyme (Fig. 3). The rate of the reaction increased by about 50% as the oxygen in the gas phase was reduced to 1%, and the results were strikingly different to those obtained with the hydroxylase from chick embryos under the same conditions. Carrying out the reaction with the *Ascaris* enzyme in an atmosphere of 1% oxygen did not affect the time course for the synthesis of $^{14}$C-hydroxyproline (Fig. 1).

A second difference was that the $K_m$ value for $\alpha$-ketoglutarate was about $4 \times 10^{-4} M$ (Fig. 4), or considerably greater than the value of $5 \times 10^{-4} M$ observed with the chick embryo enzyme (11). The $K_m$ value for $\alpha$-ketoglutarate was essentially the same at low oxygen tensions, and a value of about $7 \times 10^{-4} M$ was obtained with an atmosphere of 1% oxygen (not shown).

**Effect of Synthetic Polypeptides on Hydroxylation of Protocollagen from Chick Embryos**—A third difference from the chick...
embryo enzyme was that poly-L-proline form II did not affect the Ascaris hydroxylase (Table II). Under the conditions used, poly-L-proline II inhibited the synthesis of 14C-hydroxyproline with the chick embryo hydroxylase 65 to 97% but there was no significant effect with the Ascaris enzyme.

The Ascaris hydroxylase was similar to the chick embryo enzyme (11) in that it was not affected by addition of free L-proline to the incubation system (Table III), indicating that the Ascaris enzyme also does not interact with the free imino acid. The polytripeptides (Pro Gly Pro)n and (Gly Ala Pro)n, which are substrates for the chick embryo enzyme (8, 9), decreased the synthesis of 14C-hydroxyproline observed with 14C-protoprogelagen and the Ascaris enzyme, indicating that these polytripeptides also interact with this enzyme. With the same concentration of (Pro-Gly-Pro)n, however, the effect observed with the Ascaris enzyme was less than that observed with the chick embryo enzyme.

**Protocollagen from Ascaris as Substrate**—Proline-labeled protocollagen was prepared from the cuticle and the muscle layers of Ascaris as described above, and then the Ascaris protocollagen was incubated either with the Ascaris hydroxylase or the chick embryo hydroxylase (Table III). It should be noted that the protocollagen preparations from Ascaris were

![Fig. 3. Effect of varying the oxygen content of the atmosphere used for the enzymatic reaction. Samples were placed in Thunberg tubes, and after they had been evacuated with a water aspirator, air of various mixtures of oxygen and nitrogen were introduced at 1 atmosphere of pressure. The tubes were equilibrated at 37°C for 10 min, and then the reaction was initiated by addition of the a-ketoglutarate from the side arm. The substrate was protocollagen (40,000 dpm) from chick embryos. The other conditions were as described in text except that the reaction was carried out for only 20 min. The time of incubation and the amount of enzyme added were adjusted so that the extent of hydroxylation of the 14C-protocollagen was less than one-half the maximal value, and so that the disintegrations per min of 14C-hydroxyproline synthesized reflected the initial velocity of the reaction (see Figs. 1 and 2).](http://www.jbc.org/)

![Fig. 4. Effect of a-ketoglutarate concentration on the initial velocity for the synthesis of 14C-hydroxyproline by protocollagen hydroxylase from Ascaris. 14C-Protoprogelagen (50,000 dpm) from chick embryos was incubated for 20 min with 5.9 mg of enzyme preparation from Ascaris under the conditions described in the text and in Fig. 3.](http://www.jbc.org/)

**TABLE II**

<table>
<thead>
<tr>
<th>Enzyme source</th>
<th>Additions</th>
<th>Amount added (µg/ml)</th>
<th>14C-Hydroxyproline synthesized (dpm)</th>
<th>Relative effect (% control)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascaris</td>
<td>None</td>
<td>7.9</td>
<td>3795</td>
<td>100</td>
</tr>
<tr>
<td>Ascaris</td>
<td>Poly-L-proline II</td>
<td>1.2</td>
<td>3020</td>
<td>82</td>
</tr>
<tr>
<td>Ascaris</td>
<td>Poly-L-proline II</td>
<td>12.5</td>
<td>3020</td>
<td>104</td>
</tr>
<tr>
<td>Ascaris</td>
<td>Poly-L-proline II</td>
<td>125</td>
<td>3020</td>
<td>103</td>
</tr>
<tr>
<td>Chick embryo</td>
<td>None</td>
<td>7.9</td>
<td>7350</td>
<td>100</td>
</tr>
<tr>
<td>Chick embryo</td>
<td>Poly-L-proline II</td>
<td>1.2</td>
<td>2650</td>
<td>38</td>
</tr>
<tr>
<td>Chick embryo</td>
<td>Poly-L-proline II</td>
<td>12.5</td>
<td>1020</td>
<td>14</td>
</tr>
<tr>
<td>Chick embryo</td>
<td>Poly-L-proline II</td>
<td>125</td>
<td>200</td>
<td>3</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascaris</td>
<td>None</td>
<td>7.9</td>
<td>1070</td>
<td>100</td>
</tr>
<tr>
<td>Ascaris</td>
<td>L-Proline</td>
<td>125</td>
<td>1160</td>
<td>108</td>
</tr>
<tr>
<td><strong>Experiment 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascaris</td>
<td>None</td>
<td>7.9</td>
<td>1590</td>
<td>100</td>
</tr>
<tr>
<td>Ascaris</td>
<td>(Pro-Gly-Pro)n</td>
<td>50</td>
<td>1100</td>
<td>69</td>
</tr>
<tr>
<td>Ascaris</td>
<td>(Gly-Ala-Pro)n</td>
<td>50</td>
<td>970</td>
<td>61</td>
</tr>
<tr>
<td>Chick embryo</td>
<td>None</td>
<td>50</td>
<td>6910</td>
<td>18</td>
</tr>
<tr>
<td>Chick embryo</td>
<td>(Pro-Gly-Pro)n</td>
<td>50</td>
<td>1230</td>
<td>18</td>
</tr>
</tbody>
</table>
relatively crude, and that because of the small yields, they were not purified as extensively as the cuticle collagen preparations used in the previous study (5). After injection of \(^{14}C\)-proline into Ascaris under the conditions used here, the major part of the \(^{14}C\) incorporated into cuticle is in noncollagenous proteins, and only 20\% is recovered as \(^{14}C\)-proline and \(^{14}C\)-hydroxyproline in cuticle collagen (10). The specific activity of the protein in NaCl extracts of homogenates of cuticle and muscle layers was higher than the specific activity of the homogenates, but the NaCl extracts contained an even higher proportion of labeled noncollagenous proteins than the whole cuticle.\(^3\) The disintegrations per min of \(^{14}C\)-hydroxyproline plus \(^{14}C\)-proline in the protocollagen of the cuticle preparation were calculated with the formula

\[
\text{dpm} \quad \frac{^{14}C}{^{14}C+^{14}C} \times \frac{291}{19} \times \frac{100}{20}
\]

where \(\text{dpm} \quad \frac{^{14}C}{^{14}C+^{14}C}\) is the assayed value for \(^{14}C\)-hydroxyproline; (291:19) is the ratio of proline to hydroxyproline in normal cuticle collagen; and (100:20) is a correction factor for the ratio of proline to hydroxyproline introduced because the hydroxylation of \(^{14}C\)-proline to \(^{14}C\)-hydroxyproline is inhibited about 80\% by \(\alpha,\alpha'\)-dipyridyl under the conditions used here (10). The calculation indicated that 7050 dpm or only about 10\% of the \(^{14}C\) in the preparation was accounted for by \(^{14}C\)-hydroxyproline and \(^{14}C\)-proline in protocollagen.

The hydroxylase from Ascaris was similar to the hydroxylase from chick embryos in that it did not hydroxylate native preparations of \(^{14}C\)-proline-labeled protocollagen from Ascaris cuticle. The same protocollagen preparations, however, served as substrates for the synthesis of \(^{14}C\)-hydroxyproline with both enzymes after the preparations were heated at 100\°C for 10 min. The maximal value observed for the ratio of \(^{14}C\)-hydroxyproline to total \(^{14}C\) was only about 1.6\% with the Ascaris enzyme, and about 2.1\% with the chick embryo enzyme. These relatively low values are probably explained by the impurity of the protocollagen preparations. Under comparable conditions, denatured cuticle collagen was maximally hydroxylated from a hydroxyproline content of 19 residues per 1000 to a final value of about 80 residues per 1000 (5). If the denatured protocollagen from cuticle was hydroxylated to the same extent, and if only 10\% of the initial \(^{14}C\) in the preparations is accounted for by \(^{14}C\)-hydroxyproline and \(^{14}C\)-proline in protocollagen (above), the theoretical value for the ratio of \(^{14}C\)-hydroxyproline to total \(^{14}C\) would be about 2.6\%.

Prior denaturation was not required when \(^{14}C\)-protocollagen from the muscle layers of Ascaris was used as a substrate for the hydroxylase from either Ascaris or chick embryos (Table III). The amount of \(^{14}C\)-hydroxyproline synthesized was about 40\% greater with boiled preparations, but significant synthesis was observed with unboiled preparations of this protocollagen, and there was no significant difference between the two enzymes in this respect. The maximal value observed for the ratio of \(^{14}C\)-hydroxyproline to total \(^{14}C\) was 1.1\% with the Ascaris enzyme and 2.3\% with the chick embryo enzyme.

**Evidence on Sequence Specificity for Hydroxylation**—In an attempt to determine whether the hydroxylase from chick embryos and the hydroxylase from Ascaris hydroxylated proline in the same amino acid position of the polypeptide chain of protocollagen, protocollagen from chick embryos was hydroxylated first with one enzyme and then with the other. The purified hydroxylase from chick embryos hydroxylated the proline in the protocollagen to the extent of about 19\%, a value previously shown (14) to approximate the theoretically maximal value for the hydroxylation of proline in these protocollagen preparations. The Ascaris hydroxylase hydroxylated proline in the same chick embryo protocollagen to the extent of 13\%.\(^1\)

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\(^1\) D. Fujimoto, unpublished data.
(Table IV). Further incubation with additional enzyme and cofactors did not increase this value.

When protocollagen was incubated first with the *Ascaris* hydroxylase, and then with the chick embryo hydroxylase, the final value for the percentage formation of $^{14}$C-hydroxyproline was the same as that obtained with the chick embryo hydroxylase alone. Similarly, if the proline-labeled protocollagen was incubated first with the chick embryo enzyme and then with the *Ascaris* enzyme, the final value for percentage of $^{14}$C-hydroxyproline was the same as that obtained with incubation with chick embryo enzyme alone, suggesting that the *Ascaris* enzyme did not hydroxylate any $^{14}$C-proline not hydroxylated by the chick embryo enzyme.

**Discussion**

The presence of protocollagen proline hydroxylase has been established in a variety of vertebrate tissues (7, 15, 16), and it has been purified from chick embryos (8, 11, 12) and subsequently from the skin of newborn rats (18). The enzyme requires as cofactors or cosubstrates atmospheric oxygen, ferrous iron, α-ketoglutarate, and ascorbate (see Reference 7). The enzyme will not hydroxylate free proline or proline in tripeptides, and it is strongly inhibited by poly-L-proline form II but not by poly-L-proline form I (17). The polypeptides which will serve as substrates for the synthesis of hydroxyproline by the enzyme are biologically synthesized protocollagen with a molecular weight of 100,000 or more (14, 18); polytripeptides of the structure (Gly–X–Pro)$_n$, or (Pro–Gly–X)$_n$, in which X is proline or alanine (8, 9); or denatured collagen from the cuticle of *Ascaris* (5).

The protocollagen proline hydroxylase in *Ascaris lumbricoides* is similar to the hydroxylase from chick embryos and other vertebrate sources in that it synthesizes $^{14}$C-hydroxyproline when the same $^{14}$C-proline-labeled protocollagens are used as substrates, and it also requires atmospheric oxygen (10), α-ketoglutarate, iron, and ascorbate. The *Ascaris* enzyme did not hydroxylate protocollagen from chick embryos to the same degree as the chick embryo hydroxylase, but this is probably explained by the fact that the *Ascaris* enzyme was not purified to any significant extent. Similar low values were initially obtained with crude preparations of the chick embryo enzyme (19). Protocollagen preparations from the cuticle of *Ascaris* served as substrates only after they were boiled, suggesting that the native conformation of cuticle collagen which prevents interaction with the chick embryo enzyme (5) is also present in cuticle protocollagen, and that this conformation of the protocollagen prevents interaction with both the *Ascaris* and chick embryo hydroxylases.

The hydroxylase from *Ascaris* differs from the chick embryo enzyme in that it is partially inhibited by incubation in normal atmospheric air, and the rate of the reaction increases as the oxygen content of the gas phase is reduced to 1%. Under similar conditions, a $K_m$ value corresponding to 2.6% oxygen was previously reported (20) for the chick embryo enzyme, and a value of about 2% was observed here. The unusual dependence of initial velocity on oxygen seems to be an appropriate feature of the *Ascaris* hydroxylase, since the worm normally grows into adult form in the relatively anaerobic environment of the intestine, but the synthesis of the hydroxyproline in the collagen of both the cuticle and muscle layers requires atmospheric $\text{O}_2$ (10).

The *Ascaris* enzyme also differs in that the $K_m$ for α-keto-glutarate is about 100-fold greater than for the chick embryo enzyme, and it is possible that the requirement for α-keto-glutarate is less specific than for the chick embryo enzyme (20).

A further difference is that the *Ascaris* enzyme is not inhibited by poly-L-proline form II which competitively inhibits the chick embryo enzyme. The lack of inhibition is particularly striking, since poly-L-proline II has a high affinity for the chick embryo enzyme and the $K_m$ value for preparations with molecular weights of 12,000 to 15,000 is about 0.3 μg per ml (8, 11). The experiments in which synthetic polymers were used as inhibitors or competitive substrates for the hydroxylation of $^{14}$C-protocollagen also suggested that the *Ascaris* enzyme has less affinity for the polymer (Pro–Gly–Pro)$_n$ than the chick embryo enzyme has. Accordingly, although the *Ascaris* hydroxylase hydroxylates proline in the same protocollagens as the enzyme from vertebrate sources, different mechanisms may be involved in the binding of various polypeptide substrates to the enzyme. The experiments in which chick embryo protocollagen was hydroxylated first with one enzyme and then the other suggested that *Ascaris* enzyme is similar to the hydroxylase from chick embryos and other vertebrate sources in that it synthesizes $^{14}$C-hydroxyproline.

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**References**

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