Effects of Temperature on Conformation, Hydroxylation, and Secretion of Chick Tendon Procollagen*

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SUMMARY

When freshly isolated chick embryo tendon fibroblasts were incubated at 37°C with [14C]proline and 0.5 mM α,α'-dipyridyl, a ferrous iron chelator which inhibits prolyl hydroxylase, the labeled unhydroxylated procollagen molecules accumulated intracellularly. When pepsin digestion at 15°C was used as an enzymatic probe of conformation, these molecules were shown to be non-triple helical, indicating that hydroxylation of proline is necessary for triple helix formation at normal body temperatures. When cells which had accumulated unhydroxylated procollagen at 37°C were then incubated at 25°C, large amounts of unhydroxylated procollagen were secreted into the medium. Although these secreted molecules were sensitive to pepsin they were shown to be largely triple helical when prolyl hydroxylase was used as another probe of collagen conformation. The retained molecules were shown to be largely non-triple helical by this same test. This result suggests that triple helix formation may be necessary for normal secretion of procollagen by these cells.

When cells which had accumulated unhydroxylated procollagen at 37°C were then incubated at different temperatures between 20°C and 37°C in the presence of excess ferrous iron to reverse the inhibition of prolyl hydroxylase, the accumulated molecules were secreted and the extent of hydroxyproline formation increased as the temperature during the reversal period increased. At 20°C less than 1% of the [14C]proline in the secreted molecules was hydroxylated while at 37°C this value increased to 33%. This result implies that under some conditions the body temperature of an organism may in part regulate the hydroxyproline content of the collagen molecules.

Recent studies from several laboratories have demonstrated that collagen is first synthesized as a precursor molecule called procollagen (1-4). The procollagen molecule is composed of three polypeptide chains which are larger than the α chains of interstitial collagen because of an extension present at the NH2 terminus of each chain. These extensions are each approximately 25,000 daltons and differ from the α chain portion in their amino acid composition and conformation. The extensions are relatively poor in imino acids, relatively rich in acidic amino acids (5), and are susceptible to a number of proteolytic enzymes including pepsin (1-3). In contrast, the rest of the molecule is in a triple helical conformation, which is resistant to proteolysis by these enzymes and becomes susceptible to proteolysis only if the triple helical conformation is disrupted by heating or other means (6). Recent evidence indicates that the extensions present in the procollagen molecule are held together by disulfide bonds (7-9). The role of the extensions has not been clearly defined, but it has been postulated that they may serve to initiate triple helix formation by serving as registration peptides, they may facilitate secretion of collagen into the extracellular matrix, or they may control fibrillogenesis (10).

Although hydroxyproline occurs in all vertebrate interstitial collagens, it has been thought that it was not essential for the structural integrity of the collagen molecule, and its function has remained obscure (11). Recently we (12) and Berg and Prockop (13) have shown that hydroxyproline appears to stabilize the collagen triple helix. In further studies a series of procollagen samples, in which the hydroxyproline content varied from <1% to 44% of the total imino acids, was prepared from chick tendon fibroblasts, and the thermal stability of these procollagen preparations was then investigated by using pepsin digestion at different temperatures as an enzymatic probe of conformation (14). With the use of this technique, the denaturation temperature of the procollagen was found to be directly proportional to the hydroxyproline content. A denaturation temperature of 23.5°C was found for the unhydroxylated procollagen and 37.9°C for fully hydroxylated procollagen. These values agree well with measurements of denaturation temperatures made by optical rotation on unhydroxylated and fully hydroxylated collagen (13). These results also agree with the observation that the synthetic peptide (Pro-Hyp-Gly)11 denatures at a significantly higher temperature than (Pro-Pro-Gly)10 and that proline hydroxylation increases the thermal stability of the peptide αICB2 

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At the end of the digestion, the solutions were neutralized, sodium 0.01 M sodium phosphate, pH 7.0, 0.1% sodium dodecyl sulfate, dodecyl sulfate and mercaptoethanol were added to final concentrations of 1%, and the samples immediately placed in a boiling water bath for 2 min. The samples were then dialyzed against 0.01 M sodium phosphate, pH 7.0, 0.1% sodium dodecyl sulfate, and 0.1% mercaptoethanol and aliquots run on sodium dodecyl sulfate polyacrylamide gels or on 6% agarose columns.

**Disc Gel Electrophoresis and Agarose (Chromatography in Sodium Dodecyl Sulfate)—Disc gel electrophoresis in sodium dodecyl sulfate was performed as described previously (12) with the use of 5% acrylamide gels and half the standard amount of cross linker. The gels were immediately chilled and cut into 1.5-mm fractions in order to minimize losses, and the fractions were solubilized with 30% H2O2 and counted in a scintillation counter. Duplicate gels were prepared for containing radioactive samples and purified a and β rat tail collagens. These gels were stained with Coomassie blue and the radioactive peaks located relative to these markers. The positions of the radioactive peaks were identical in the stained and unstained gels when corrected for gel swelling during destaining. Radioactively labeled collagen samples were chromatographed on sodium dodecyl sulfate-agarose columns (Bio-Gel P-200 to 400 mesh, Bio-Rad Laboratories, Rockville Centre, N. Y.) as described previously (3).

**Preparation of Prolyl Hydroxylase and U, Lhydroxylated Procollagen—Prolyl hydroxylase was purified from 13 day old chick embryos as described by Halmo et al. (25). The purification was carried through the DEAE-cellulose chromatography step, and the final enzyme preparation had a specific activity of 45 units per mg of protein when measured with 125 pg per ml of (Pro-Gly-Pro), molecular weight 2400, as substrate (purchased from Miles Laboratories, Inc., Kankakee, Ill.). One unit is defined as that amount of enzyme required to synthesize 1 μg of hydroxyproline in 1 hour at 37°C under conditions in which the concentrations of all other cofactors are saturating. Unhydroxylated [14C]proline-labeled procollagen was prepared by incubating isolated fibroblasts with 0.5 mM α,α'-dipyridyl for 5 hours and then extracting the [14C]procollagen from the cells with 0.5 M acetic acid at 4°C (21). The extract was centrifuged at 12,000 × g for 15 min, and the supernatant was then dialyzed exhaustively against 0.5 M acetic acid. This dialyzed material served as substrate for the hydroxylase. Conditions of the hydroxylation reaction are described in Table 1.

**RESULTS**

**Conformational State of Unhydroxylated Procollagen Retained Intracellularly at 37°C** When the cells were incubated with [14C]proline and either 0.5 mM α,α'-dipyridyl or 1 μM colcemide for 2 hours, the procollagen synthesized by the cells was secreted at a markedly reduced rate relative to control cells, and the [14C]procollagen accumulated intracellularly. In the case of the α,α'-dipyridyl-treated cells, the [14C]procollagen contained essentially no [14C]hydroxyproline while the [14C]procollagen from the colcemide-treated cells was fully hydroxylated (28). In order to avoid changes in the conformation of the molecules, the cell suspensions were quickly centrifuged at the end of the incubation and aliquots of the resuspended cells immediately incubated with or without pepsin. The digest mixtures were neutralized and subjected to electrophoresis on sodium dodecyl sulfate polyacrylamide gels (Fig. 1). The fully hydroxylated collagen synthesized by the colcemide-treated cells was recovered as procollagen when pepsin was omitted from the digestion. After pepsin digestion, 85% of the radioactivity originally in the procollagen peak was recovered as α chains, thus indicating that it was predominantly triple helical. In contrast, less than 7% of the radioactivity originally in the unhydroxylated procollagen was recovered as α chains after pepsin digestion, and smaller molecular weight products were observed near the tracking dye. This result demonstrates that the unhydroxylated procollagen within the cells at 37°C was not triple helical.

It has been proposed that the additional amino acid residues at the NH2 termini, in which the chains are linked together by disulfide bonds, may serve as a nucleation site for triple helix formation. It was possible, therefore, that α,α'-dipyridyl could have interfered with the formation of these disulfide link-
Table I

<table>
<thead>
<tr>
<th>Substrate</th>
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<th>25°C</th>
<th>37°C</th>
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<tr>
<td></td>
<td>Total dpm X 10^4</td>
<td>Degree of hydroxylation</td>
<td>Total dpm X 10^4</td>
</tr>
<tr>
<td>Denatured unhydroxylated procollagen</td>
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<td></td>
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<tr>
<td>Secreted</td>
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<tr>
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<td>13.7</td>
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</tr>
<tr>
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<td>7.2</td>
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</tr>
<tr>
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<td>97.6</td>
<td>20.1</td>
<td></td>
</tr>
<tr>
<td>Denatured unhydroxylated procollagen</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Secreted</td>
<td>177.9</td>
<td>0.2</td>
<td></td>
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</tbody>
</table>

Values are 100 X [14C]hydroxyproline per total [14C] collagen.

Prepared as described under "Materials and Methods." The degree of hydroxylation was <1%.

Fig. 1. Polyacrylamide sodium dodecyl sulfate gel electrophoresis of intracellular procollagen after incubation with or without pepain. Aliquots of cells which had been incubated with 1 mM colcemide, A, or 0.5 mM α,α'-dipyridyl, B, and [14C]procollagen for 2 hours were then incubated with or without 100 μg per ml of pepain at pH 2.0 for 6 hours at 15°C. At the end of the digestion the solutions were neutralized, prepared for electrophoresis, run on 7% polyacrylamide gels, and the gels fractionated and counted as described under "Materials and Methods." A, incubation with pepain; O—a, without pepain; f, rat tail α chains; $β$, rat tail β chains; $α$, tracking dye.

Cell incubation (25°C)

at or below 25°C at neutral pH (30, 31). We therefore incubated cells, containing accumulated unhydroxylated procollagen, at 25°C or 37°C in order to compare the rate at which they secreted the unhydroxylated molecules at the two temperatures (Fig. 3). When the cells were incubated at 25°C there was a progressive secretion of the accumulated unhydroxylated molecules so that after 3 hours 41% of the nondialyzable radioactivity was found in the medium (Fig. 3). When the cells were incubated at 37°C, the secretion was irregular, and only 26% was found in the medium. Corresponding to the increase in radioactivity in the medium at 25°C, there was a decrease in the radioactivity found intracellularly. At 37°C the secreted radioactivity was slightly less than the corresponding intracellular decrease.

These results suggested that differences might exist between the structure of the protein secreted at 25°C and 37°C, and aliquots of the secreted proteins were, therefore, subjected to electrophoresis on polyacrylamide gels. The results (Fig. 4) demonstrated that over 60% of the radioactivity in the proteins secreted at 25°C was recovered in the molecular weight range of 125,000. However, when the secreted proteins were digested with pepain at 15°C before electrophoresis, only lower molecular weight peptides were recovered. This result indicates that although the chains had a molecular weight equal to that of procollagen, they were either completely denatured or contained regions of local collagens and in this way impaired triple helix formation. Aliquots of cells incubated as described above were therefore prepared for gel electrophoresis in sodium dodecyl sulfate containing no mercaptoethanol and 0.1 mM iodoacetamide to scavenge any possible reducing agents present in the sodium dodecyl sulfate itself (29). The results shown in Fig. 2 demonstrate that when reducing agents were absent only about 25% of the unhydroxylated and 18% of the fully hydroxylated intracellular procollagen was found as single chains, indicating that α,α'-dipyridyl did not interfere with disulfide formation.

Secretion of Unhydroxylated Procollagen at 25°C—Recent experiments using prollyl hydroxylase have indicated that unhydroxylated procollagen is largely in a triple helical conformation only immediately prior to incubation with the enzyme was used as a substrate for prollyl hydroxylase.

Immediately prior to incubation with the enzyme was used as a substrate for prollyl hydroxylase.

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FIG. 2. Polyacrylamide sodium dodecyl sulfate gel electrophoresis of intracellular procollagen prepared for electrophoresis in the presence or absence of reducing agent. Aliquots of cells which had been incubated with 0.5 mM α,α'-dipyridyl, A, or 1 μM colcemide, B, were resuspended in 0.01 M sodium phosphate, pH 7.0, 1% sodium dodecyl sulfate containing either 1% mercaptoethanol or 0.1 M iodoacetamide, and then placed in a boiling water bath for 2 min. The samples were then dialyzed against 0.01 M sodium phosphate, pH 7.0, 1% sodium dodecyl sulfate containing either 0.1% mercaptoethanol or 0.1 M iodoacetamide. Electrophoresis was then carried out as described in Fig. 1 and under "Materials and Methods," except that it was continued for 1 hour longer in order to allow the higher molecular weight molecules in the nonreduced samples to enter the gel further.

**O-O, prepared for electrophoresis with 1% mercaptoethanol; O-O, prepared with 0.1 M iodoacetamide.**

denaturation, sensitive to pepsin, which led to degradation of the collagen. Similar digestion of the intracellular proteins demonstrated that they were also sensitive to pepsin. Our findings with the proteins secreted at 37° agreed with previous results showing that only degraded fragments were found in the medium (21).

**Ability of Unhydroxylated Procollagen Isolated from Cells Incubated at 25° or 37° to Serve as Substrate for Prolyl Hydroxylase**—
Previous experiments have indicated that prolyl hydroxylase can only hydroxylate regions of the collagen molecule which are not triple helical (30, 31). We, therefore, tested the ability of the unhydroxylated proteins isolated from the medium and cells incubated at 25° or 37° to serve as substrate for prolyl hydroxylase (Table I). The proteins secreted at 25° had a degree of hydroxylation of 5.9% after incubation with excess prolyl hydroxylase and this value increased to 19.9% when the hydroxylation was carried out at 37°. In contrast, the proteins secreted at 37° were relatively poor substrates and had a degree of hydroxylation of 7.2% when hydroxylated at 25° and 11.1% when hydroxylated at 37°. The proteins retained intracellularly at 25° or 37° also demonstrated a significant difference in their ability to serve as substrate at the two hydroxylation temperatures of 25° and 37°. The proteins in the cells incubated at 25° were hydroxylated to about 14 to 15%, at both hydroxylation temperatures. In contrast, the proteins in the cells incubated at 37° were hydroxylated to 20.1% at 25° and to 30.7% at 37°. We have interpreted these findings to mean that molecules which are largely triple helical are secreted at 25° and that molecules which are largely non-triple helical are retained intracellularly at both 25° and 37°. These results are considered more extensively under "Discussion," but they suggest that triple helicity may be required for normal secretion of procollagen.

**Intracellular Hydroxylation at Different Temperatures of Unhydroxylated Procollagen Accumulated at 37°**—When extracted unhydroxylated procollagen or collagen was incubated with prolyl hydroxylase at various temperatures, the amount of hydroxyproline which could be formed increased sharply between 20° and 30° (30, 31). In order to determine whether temperature had a similar effect on the intracellular hydroxylation of accumu-
labeled procollagen independently of the effect of temperature on the rate of protein synthesis, the following experiments were performed. Unhydroxylated procollagen was accumulated intracellularly at 37° by incubating cells with 0.5 mM α, α'-dipyridyl for 1 hour. The cells were then washed and resuspended in fresh medium containing 25 μg per ml of cycloheximide to stop protein synthesis and 1 mM FeSO₄ to reverse the inhibition of prolyl hydroxylase. Aliquots of the cell suspension were then incubated at 20°, 25°, 30°, or 37° for 3 hours. At hourly intervals, samples were removed and the degree of hydroxylation of the intracellular and secreted proteins was determined. The results from this experiment (Table II) show a pronounced dependence of the degree of hydroxylation on temperature. For example, at 20° less than 2% of the radioactivity in the complete system (intracellular plus secreted) was in hydroxyproline, while at 37° this value was approximately 14%. Intermediate temperatures resulted in intermediate values. Note also that for a given temperature these values appeared to represent maximal attainable values, since they did not change appreciably between the 2nd and 3rd hours of incubation. Under the conditions of this experiment, a progressively greater fraction of the radioactivity was secreted into the media as the temperature during the reversal period was increased.

In order to determine the degree of hydroxylation of the procollagen itself, aliquots from the 3-hour time point of the secreted and intracellular proteins were chromatographed on agarose A-5 columns in 0.1% sodium dodecyl sulfate. Chromatograms of the media proteins are presented in Fig. 5. They demonstrate that a large fraction of the labeled proteins eluted in the position of procollagen at all temperatures, although a significant fraction was recovered as degraded fragments at 37°. [¹⁴C]Hydroxyproline analyses of the procollagen peaks (Table III) showed a marked increase in the degree of hydroxylation as the temperature increased between 20° and 37°. A similar effect of temperature was obtained when the procollagen retained intracellularly was examined (chromatograms not shown, but see Table III).

**Discussion**

It was previously reported (21) that unhydroxylated procollagen which was retained intracellularly was triple helical since it was resistant to pepsin digestion at 15° and formed normal segment-long spacing aggregates at 4°. However, more recent experiments (12–16), which demonstrated that hydroxyproline is crucial to the thermal stability of collagen and procollagen, suggested that unhydroxylated procollagen might not be triple helical within the cell at 37°. In the present experiments the conformation of the intracellular unhydroxylated procollagen was examined by pepsin digestion under conditions designed to mini-

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**Table II**

<table>
<thead>
<tr>
<th>Cell incubation temperature</th>
<th>Intracellular Secreted</th>
<th>Secreted</th>
<th>Intracellular + Secreted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total dpm X 10⁻²</td>
<td>Degree of hydroxylation°</td>
<td>Total dpm X 10⁻²</td>
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<tr>
<td>Zero time</td>
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<tr>
<td>20°</td>
<td>21.9</td>
<td>0.4</td>
<td>3.9</td>
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<tr>
<td>1 hour</td>
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<tr>
<td>25°</td>
<td>20.0</td>
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<td>3.8</td>
</tr>
<tr>
<td>1 hour</td>
<td>19.3</td>
<td>4.2</td>
<td>4.4</td>
</tr>
<tr>
<td>3 hours</td>
<td>15.2</td>
<td>5.1</td>
<td>5.1</td>
</tr>
<tr>
<td>30°</td>
<td>20.3</td>
<td>8.3</td>
<td>4.2</td>
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<tr>
<td>1 hour</td>
<td>15.7</td>
<td>9.6</td>
<td>5.5</td>
</tr>
<tr>
<td>3 hours</td>
<td>17.2</td>
<td>8.0</td>
<td>6.9</td>
</tr>
<tr>
<td>37°</td>
<td>19.8</td>
<td>14.2</td>
<td>5.7</td>
</tr>
<tr>
<td>1 hour</td>
<td>17.7</td>
<td>15.5</td>
<td>7.3</td>
</tr>
<tr>
<td>3 hours</td>
<td>15.6</td>
<td>15.6</td>
<td>8.7</td>
</tr>
</tbody>
</table>

° Values are 100 x [¹⁴C]hydroxyproline per total [¹⁴C].

**Fig. 4. Polyacrylamide gel electrophoresis of unhydroxylated procollagen from cells incubated at 25°.** Cells were incubated as described in Fig. 3, and aliquots of the secreted and intracellular proteins from the 2-hour time point were incubated with or without 100 μg per ml of pepsin for 6 hours at 15°. The incubation mixtures were prepared for electrophoresis and then subjected to electrophoresis as described in Fig. 1. A, secreted proteins; B, intracellular proteins; •—•, incubation with pepsin; O—O, without pepsin. 1, rat tail β chains; 2, rat tail α chains; 3, tracking dye.
Prolonged dialysis period at 4°.

apparently reformed native molecules during the extraction and disulfide bond formation by Q, a'-dipyridyl. In the earlier report of the two types of molecules cannot be due to prevention of by disulfide linkages (Fig. 2), the difference in the conformation prevented secretion of whole procollagen molecules at 37°.

The experiments using denatured procollagen proved that completely denatured molecules could be fully hydroxylated at 25°. Control experiments using denatured procollagen proved that completely denaturated molecules could be fully hydroxylated at 25°. The results with the secreted unhydroxylated proteins were fairly clear. The unhydroxylated procollagen secreted at 25° was hydroxylated to a much greater extent when incubated with prolyl hydroxylase as another probe of collagen conformation.

The reason for the difference between the cultured fibroblasts and the freshly isolated fibroblasts is not clear. Perhaps the secretory ability of the freshly isolated cells may have been impaired by the proteolytic digestion required to free them from the extracellular matrix, or alternatively the ability of the fibroblasts in culture to discriminate triple helical from non-helical molecules may be impaired. The present results indicate that the inability of the isolated tendon cells to secrete unhydroxylated procollagen can be overcome by incubating the cells at 25°, since large amounts of unhydroxylated procollagen were secreted at 25° and essentially none at 37°. This is all the more remarkable since we would expect all cellular functions including secretory ability to be considerably slower at 25° than at 37°.

The unhydroxylated molecules secreted at 25° were not perfectly triple helical, however, since they were sensitive to pepsin. Pepsin digestion, as presently used, is a stringent test of triple helicity since a single disruption of the triple helix probably leads to digestion. Recent experiments studying the ability of unhydroxylated procollagen to serve as a substrate for prolyl hydroxylase as a function of temperature have strongly indicated that only procollagen molecules which are not in a triple helical conformation can be hydroxylated (30, 31). The results with the secreted unhydroxylated proteins were analyzed.

![Agarose gel chromatography of secreted procollagen](image)

**Fig. 5.** Agarose gel chromatography of secreted procollagen which was hydroxylated intracellularly at different temperatures. Cells were incubated as described in Table II except that 2 μCi per ml of [14C]proline was used. At the end of the 3-hour reversal period at the different temperatures, the secreted proteins were prepared for chromatography as described under "Materials and Methods." One hundred thousand counts per min of each sample were chromatographed on a column (1.5 × 85 cm) of 6% agarose (Bio-Gel A-5m, 200 to 400 mesh, Bio-Rad Laboratories, Rockville Centre, N. Y.). The fraction size was 2.0 ml. A, 20°; B, 25°; C, 30°; D, 37°; 1, marker β rat tail collagen; 2, marker α rat tail collagen.

mize the opportunity for conformational changes in the protein. Under these conditions 93% of the unhydroxylated molecules were digested while only 15% of the hydroxylated intracellular molecules were digested. By this test then the unhydroxylated molecules were largely not triple helical while the hydroxylated molecules were triple helical. Since most of both the unhydroxylated and hydroxylated chains appeared to be held together by disulfide linkages (Fig. 2), the difference in the conformation of the two types of molecules cannot be due to prevention of disulfide bond formation by α,α'-dipyridyl. In the earlier report (21) the unhydroxylated collagen was extracted from the cells and apparently reformed native molecules during the extraction and prolonged dialysis period at 4°.

<table>
<thead>
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<th>Temperature of hydroxylation</th>
<th>Intracellular</th>
<th>Secreted</th>
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<tbody>
<tr>
<td></td>
<td>Degree of hydroxylation</td>
<td>Degree of hydroxylation</td>
</tr>
<tr>
<td>20°</td>
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<td>25°</td>
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</tr>
<tr>
<td>37°</td>
<td>33.0</td>
<td>32.7</td>
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</table>

*Values are 100 × [14C]hydroxyproline per total 14C.*
intrapcellularly at 25° were equally good substrates for hydroxylation at 25° and 37°, they appeared to be less triple helical than the secreted ones. Although the findings with the molecules retained at 37° qualitatively agree with the interpretation that they were largely non-triple helical, one quantitative inconsistency exists. That is the fact that these molecules were somewhat better substrates at 37° than at 25°. We have no certain explanation for this observation, but it is possible that limited helical regions in fact existed intracellularly or were formed during the work up of the cells at room temperature prior to the hydroxylation at 25° or during the hydroxylation at 25° itself. Nevertheless, by the criterion of their ability to be hydroxylated, the retained intracellular molecules were largely non-helical, and it is possible that the proper conformation may be essential for rapid secretion of procollagen by these cells.

When unhydroxylated procollagen was incubated with purified prolyl hydroxylase in a cell-free system at different temperatures, the amount of hydroxyproline which could be formed increased sharply between 26° and 30°, provided the substrate was not denatured prior to incubation with the enzyme (30, 31). These experiments suggested that under certain conditions temperature increased during the reversal period. One possible explanation of these results is that partial triple helix formation might regulate the degree of hydroxylation achievable intracellularly. The present experiments, in which the process of protein synthesis has been dissociated from hydroxylation, and the accumulated unhydroxylated procollagen then hydroxylated intracellularly by reversal of inhibition of prolyl hydroxylase at different temperatures support the possibility that such regulation can take place. Kinetic experiments (Table II) at the different temperatures showed that the hydroxylation reaction was essentially complete 2 hours after the inhibition of prolyl hydroxylase was reversed. Hydroxyproline determinations on the isolated procollagen (Table III) showed that the degree of hydroxylation of the molecules increased markedly when the temperature increased during the reversal period. One possible explanation of these results is that partial triple helix formation occurs more readily at lower temperatures, and the triple helical regions of the molecule cannot be hydroxylated further, resulting in lower degrees of hydroxylation. These experiments suggest that under some conditions the body temperature of an organism may in part regulate the hydroxyproline content of collagen molecules.

Acknowledgments—We thank Miss Sandy Schnee and Mrs. Marie Pratt for superior technical assistance.

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