Inhibition of the Assembly and Secretion of Procollagen by Incorporation of a Threonine Analogue, Hydroxynorvaline*

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Hydroxynorvaline (DL-α-amino-β-hydroxyvaleric acid) was shown to competitively inhibit the activation of threonine and valine when tested with tRNA and synthetases prepared from whole chick embryos. However, the hydroxynorvaline was transferred only to threonyl-tRNA and not valyl-tRNA. The hydroxynorvaline had no effect when tested with other amino acids. The $K_m$ for threonine was 25 μM and the $K_i$ for hydroxynorvaline was 181 μM. When fibroblasts from embryonic chick tendons were incubated with embryonic chick tendons were incubated with $[^3H]$threonine and increasing concentrations of hydroxynorvaline, there was a progressive decrease in the incorporation of $[^3H]$threonine so that at 1 mM hydroxynorvaline the incorporation into nondialyzable protein was 26% of the control value. A much smaller decrease in the incorporation of other radioactive amino acids was observed. When the cells were incubated with $[^3H]$proline and 1 mM hydroxynorvaline, the labeled procollagen containing hydroxynorvaline accumulated intracellularly and very little was secreted. Control experiments demonstrated that free hydroxynorvaline did not inhibit the secretion of unsubstituted procollagen.

Although the individual pro α chains containing hydroxynorvaline were of normal molecular weight (125,000) and hydroxyproline content, only about 50% of this intracellularly retained procollagen was triple helical within the cell at 37° as measured by sensitivity to pepsin digestion. Also only approximately 50% of the pro α chains were disulfide-linked to form triple stranded molecules as compared to greater than 85% linkage in unsubstituted procollagen. We postulate that incorporation of hydroxynorvaline alters the conformation of the propeptide extension sufficiently so that: (a) normal assembly of disulfide-linked, triple helical molecules is reduced and (b) assembled triple helical molecules are not properly recognized by the secretory mechanism.

It is now well established that collagen is first synthesized as a precursor molecule called procollagen, in which the individual polypeptide chains are larger than the α chains of interstitial collagen because of an extension of the NH$_2$ terminus of each chain (1–4). 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 and hydroxyamino acids (5, 6), 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 (7). The extensions present in the procollagen molecule are held together by disulfide bonds (8–10). 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 (11).

The procollagen molecule undergoes a number of modifications within the cell before secretion. These include hydroxylation of some of the prolyl and lysyl residues, and glycosylation of some of the hydroxylysyl residues. When the hydroxylation of proline and lysine was inhibited in embryonic tibiae (12) and fibroblasts (13, 14), unhydroxylated procollagen was secreted at a markedly reduced rate and accumulated intracellularly. It now appears that hydroxyproline stabilizes the collagen triple helix (15–19), and that unhydroxylated collagen is found within cells at 37° in a random coil conformation. Since triple helix formation normally occurs intracellularly before collagen is secreted (20), these observations suggested that triple helix formation may be required for normal secretion. This hypothesis is supported by the finding that unhydroxylated procollagen molecules can be secreted by cells incubated at lower temperatures at which the molecules are largely triple helical (21).

When tibiae (22–25) or fibroblasts (26–29) were incubated with analogues of proline or lysine, the analogues were incorporated into collagen and the analogue-containing collagens were

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secreted more slowly than normal collagen. The collagens containing proline analogues were largely nontriple helical at 37 °C within cells, but some of the intracellularly retained procollagen molecules containing the lysine analogue appeared to be triple helical. However, by and large, these experiments with the analogues supported the concept that the triple helical conformation was essential for normal secretion. In these experiments no attempt was made to examine possible alterations in the conformation of the propeptide extension by incorporation of the analogues.

In the present study we show that α-amino-β-hydroxyvaleric acid 1 specifically replaces threonine in procollagen synthesized by chick fibroblasts. The procollagen chains containing the analogue were of normal molecular weight and hydroxyproline content but formed interchain disulfide linkages less efficiently and were secreted much more slowly than normally. We postulate that incorporation of hydroxynorvaline alters the conformation of the propeptide portion sufficiently so that: (a) normal assembly of triple stranded chains is reduced, and (b) assembled triple helical molecules are not properly recognized by the secretory mechanism.

EXPERIMENTAL PROCEDURES

Materials—Radioactive amino acids were purchased from New England Nuclear (Boston, Mass.). Crystalline pepsin, colcemide, cycloheximide, sodium dodecyl sulfate, and mercaptoethanol 1 were purchased from Sigma Chemical Co. (St. Louis, Mo.). Reagents used for acrylamide disc gel electrophoresis were purchased from Eastman Kodak Co. Hydroxyynorvaline was purchased from Calbiochem. It was analyzed by chromatography on the long column of a Jeolco amino acid analyser. The tRNA in a position close to serine, and by thin layer chromatography. A small amount of contaminants was observed but they collectively constituted less than 0.1% of the total ninhydrin-positive material and the hydroxyynorvaline was used without further purification.

Preparation of Transfer RNA, Activating Enzymes, and Assay of Effect of Hydroxyynorvaline on Amino Acid Activation—Whole 17-day-old chick embryos were homogenized in a Waring Blender in 0.1 M Tris-HCl, pH 7.8, 0.15 M NaCl, 10 mM MgCl₂, and 1 mM EDTA, containing 0.4 mg/ml of bentonite. The homogenate was centrifuged at 15,000 × g for 15 min. The supernatant was removed and 80% redistilled phenol was added to this aqueous phase. The mixture was shaken for 15 min at room temperature. The phases were then separated by centrifuging for 15 min in a Sorvall centrifuge and the aqueous phase was added to this aqueous phase. The mixture was filtered through a little glass wool and washed with a small volume of 0.5 N NaCl, 0.1 M Tris-HCl, pH 7.4, 10 mM MgCl₂, and 1 mM EDTA, containing 0.4 mg/ml of bentonite. The homogenate was centrifuged at 15,000 × g for 15 min in a Sorvall centrifuge and the supernatant was added to 1.0 M Tris-HCl, pH 7.4, containing 10 mM MgCl₂, 1 mM EDTA, and 0.4 mg/ml of bentonite. The solution was then made 1 M with respect to NaCl and allowed to stand in an ice bath for 2 hours. The precipitate which formed containing ribosomal RNA was removed by centrifugation and the supernatant was diluted so that the NaCl concentration was 0.3 M and applied to a DEAE-cellulose column (1.0 × 4.0 cm). After passage of 2 column volumes of 0.1 M Tris-HCl, pH 7.4, containing 0.3 M NaCl, 10 mM MgCl₂, and 1 mM EDTA, the tRNA was eluted in increasing the NaCl concentration to 0.7 M. The absorbance at 260 mm of each fraction was read and peak fractions were pooled and precipitated with 2 volumes of cold ethanol. The tRNA was redissolved in 0.1 M Tris-HCl, pH 9.8, with 10 mM MgCl₂, and incubated for 30 min at 37 °C to remove any attached amino acids from the tRNA. The tRNA was then precipitated by the addition of 2 volumes of cold ethanol, centrifuged, redissolved in distilled H₂O, and lyophilized. Prior to use, it was redissolved in 10 mM MgCl₂.

Seventeen-day-old embryos were used for the preparation of amino

1 The abbreviations and trivial names used are: hydroxyynorvaline, DL-α-amino-β-hydroxyvaleric acid, allo-free; Heps, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid.

RESULTS

Competition of Amino Acid Activation by Hydroxyynorvaline—Hydroxyynorvaline showed competitive kinetics in the charging of threonine when tested with purified tRNA and a relatively crude preparation of activating enzyme (Fig. 1). The
neutral pH and low temperature and varies with the amino acid and specific conditions (34).

Inhibition of Incorporation of Radioactive Amino Acids by Hydroxynorvaline—In order to confirm the specific replacement of threonine by hydroxynorvaline and to study the effects of this replacement in procollagen, chick fibroblasts were incubated with radioactive amino acids in the presence or absence of hydroxynorvaline for 2 hours. Increasing concentrations of the analogue progressively diminished the incorporation of $[^{1}C]threonine, so that at 1 mM hydroxynorvaline the incorporation of $[^{1}C]threonine into nondialyzable protein was only 26% of the control value and at 4 mM it was 6% (Fig. 2). A much smaller effect on the incorporation of other radioactive amino acids was observed. After 1 hour of incubation in the presence of 1 mM hydroxynorvaline, the incorporation ranged from 59 to 73% of the control values for the other amino acids studied, compared to 35% for threonine (Table III). The inhibition of incorporation of other amino acids besides threonine was independent of the concentration of hydroxynorvaline above 1 mM in contrast to the results with threonine in which there was a progressive inhibition of incorporation. These findings, in addition to demonstrating the relatively specific effect on threonine incorporation, suggested that protein synthesis was being inhibited and that this inhibition increased with incubation time. This effect was studied in more detail using $[{}^{14}C]proline$ (see below).

Inhibition of Secretion of Procollagen Containing Hydroxynorvaline—When the fibroblasts were incubated with 1 mM hydroxynorvaline and $[{}^{14}C]proline$, two major differences were observed from the control: (a) there was a pronounced inhibition of secretion of the procollagen which was synthesized, and (b) there was a significant inhibition of total

\[ V = \frac{1}{K_m} \times \frac{1}{[\text{amino acid}]} \]

$K_m$ for threonine was 25 $\mu M$ and the $K_i$ for hydroxynorvaline was 181 $\mu M$. Hydroxynorvaline was not competitive with any other amino acid tested except valine (Table I). In order to determine whether hydroxynorvaline was transferred to threonyl- and/or valyl-specific tRNA, the following experiment was performed. Aliquots of tRNA were incubated under conditions of maximal acylation with either $[^{14}C]hydroxynorvaline$, valine, threonine, or no amino acid. The tRNA was then dialyzed and aliquots were reacylated with either $[^{3}H]valine$ or $[^{3}H]threonine$. If the tRNA were maximally acylated and remained acylated during the dialysis period, then no radioactive activity would be incorporated. The tRNA sample which was incubated without any $[^{14}C]$-amino-acid served as a control for maximal acylation with the radioactive amino acids. The results of this experiment demonstrated that hydroxynorvaline was transferred only to threonyl-specific tRNA since the analogue had no effect on the incorporation of $[^{3}H]valine$ and was as effective as threonine in inhibiting the incorporation of $[^{14}C]threonine$ (Table II). In the case of threonine, apparently a significant fraction of the tRNA which was charged with nonradioactive amino acid decylated during the dialysis and recovery of the tRNA. Decylation has been observed at neutral pH and low temperature and varies with the amino acid and specific conditions (34).

**Table I**

| Amino Acid | Rate control | Rate + 1 mM Hydroxynorvaline | (Rate + Hydroxynorvaline/rate control) x 100 |
|------------|--------------|-----------------------------|---------------------------------------------|
| Alanine    | 54           | 52                          | 96                                          |
| Glutamate  | 94           | 100                         | 106                                         |
| Isoleucine | 8            | 9                           | 113                                         |
| Leucine    | 9            | 9                           | 100                                         |
| Lysine     | 240          | 240                         | 100                                         |
| Methionine | 32           | 32                          | 100                                         |
| Phenylalanine | 20         | 19                          | 95                                          |
| Proline    | 84           | 88                          | 105                                         |
| Serine     | 130          | 116                         | 89                                          |
| Threonine  | 14           | 4                           | 29                                          |
| Valine     | 30           | 13                          | 43                                          |

$\text{Sample charging conditions } \times 10^2$ (\(\mu \text{MOLERE} / \text{M} / \text{MIN}\))

| Sample charging conditions | Incorporation |
|-----------------------------|---------------|
| No amino acid, then $[^{14}C]threonine$ | 3870 ± 230 |
| $[^{14}C]threonine$, then $[^{3}H]threonine$ | 1970 ± 82 |
| Hydroxynorvaline, then $[^{14}C]threonine$ | 1670 ± 59 |
| No amino acid, then $[^{3}H]valine$ | 8210 ± 110 |
| $[^{3}H]valine$, then $[^{14}C]valine$ | 2200 ± 151 |
| Hydroxynorvaline, then $[^{3}H]valine$ | 8010 ± 64 |

TABLE II

Test of charging of threonyl- and valyl-specific tRNA by hydroxynorvaline

Aliquots of tRNA were charged using the assay conditions described in the text but with 1.25 mg/ml of tRNA and a reaction time of 30 min for maximal charging with either $50 \mu M$ $[^{14}C]threonine$, $50 \mu M$ $[^{1}C]valine$, 10 $\mu M$ hydroxynorvaline, or no amino acid. The samples were then dialyzed at 4° against 100 volumes of 0.13 M Hepes, pH 7.4, for 6 hours with three changes. Aliquots of the dialyzed samples were then charged for 30 min in duplicate with either $[^{1}C]valine$ or $[^{1}C]threonine$. In this experiment the background values observed when either tRNA or activating enzyme was omitted were less than 5% of that observed in the complete reaction mixture. These background values were the same in the presence or absence of hydroxynorvaline. Averages are presented.

Incubations were carried out in duplicate under the assay conditions described in the text. The values agreed within 5% and the averages are presented. All amino acids were uniformly labeled and tested at a concentration of 10 $\mu M$ with and without 1 mM hydroxynorvaline.
incorporation into the system and this inhibition increased with incubation time (Fig. 3). In the control after 90 min of incubation, 49% of the incorporated radioactivity was found secreted into the medium. Approximately 90% of this secreted label has been shown previously to be in the form of procollagen (31). In the presence of the analogue, only 13% of the incorporated radioactivity was in the medium. After 30 min of incubation, total incorporation in the presence of the analogue was 75% of the control while after 90 min it declined to 49%.

In order to demonstrate that hydroxynorvaline had to be incorporated into procollagen in order to inhibit secretion, an experiment was designed so that hydroxynorvaline was present either during the synthesis of the procollagen but absent during the period of secretion or it was absent during synthesis but present during the period of secretion. The results of this experiment, presented in Table IV, clearly demonstrate that hydroxynorvaline exerts its effect only when it is present during the synthesis of procollagen and that free hydroxynorvaline has no effect on the secretion of unsubstituted procollagen.

**Molecular Weight, Conformation, and Hydroxyproline Content of Procollagen Containing Hydroxynorvaline**—In order to estimate the molecular weight of the individual procollagen chains synthesized in the presence of hydroxynorvaline and to determine whether they were in a triple helical conformation, aliquots of cells containing procollagen labeled with $[^3]C^3$ proline and synthesized in the presence of 1 mM hydroxynorvaline were incubated with or without pepsin at 15°. The digest mixtures were neutralized and then chromatographed on agarose A-5 columns in sodium dodecyl sulfate (Fig. 4). When pepsin was omitted from the digestion, the analogue-containing procollagen was recovered in a position identical with that of control procollagen with an approximate molecular weight of 125,000 (control not shown). The procollagen peaks were recovered and analyzed for their $[^3]C^3$ hydroxyproline content. The degree of hydroxylation of the procollagen peak containing the hydroxynorvaline was 37.6% compared to a value of 35.6% for control procollagen obtained from cells incubated with colcemide to cause intracellular retention. When the analogue-containing proteins were digested at 15° with pepsin before chromatography, the quantity of radioactivity recovered as $\alpha$ chains was only about 53% of the amount originally found as pro $\alpha$ chains. In cells incubated under control conditions, greater than 85% of the intracellular procollagen was resistant to pepsin and recovered as $\alpha$ chains (21). These results suggested that a significant fraction of the intracellular hydroxynorvaline-containing procollagen chains was not triple helical within the cell.

In order to examine the stability of the procollagen in more detail, $[^3]C^3$ proline-labeled procollagen was extracted from cells incubated with 1 mM hydroxynorvaline and aliquots of this procollagen were incubated with pepsin at different temperatures up to 37°. As demonstrated in Table V, the fraction of procollagen resistant to pepsin remained constant at approximately 56% up to 37°. These results indicated that a majority of the intracellular hydroxynorvaline-containing procollagen chains were found in triple helical molecules which were equal in thermal stability to unsubstituted procollagen.

The finding of two classes of procollagen chains, pepsin-sensitive and pepsin-resistant, suggested that incorporation of hydroxynorvaline might inhibit disulfide bond formation and hence decrease the efficiency of assembly of procollagen chains into triple helical molecules. To test this possibility, intracellular $[^3]C^3$ proline-labeled procollagen synthesized in the presence of hydroxynorvaline was subjected to electrophoresis on sodium dodecyl sulfate polyacrylamide gels in the presence or absence of a reductant. In the presence of a reductant, procollagen chains migrate with an approximate molecular weight of 125,000 while in the absence of a reductant the linked chains migrate much more slowly. By comparing the quantity of radioactivity recovered as individual pro $\alpha$ chains in the presence and absence of a reductant, we have found that only one-half of the analogue-containing procollagen chains were disulfide-linked, in contrast to unsubstituted procollagen in
FIG. 3. Effect of hydroxynorvaline on the synthesis and secretion of procollagen. Chick fibroblasts were incubated with or without 1 mM hydroxynorvaline for 20 min and then [14C]proline was added to a final concentration of 2 µCi/ml and the incubation continued for 30 min. At that time the cells were centrifuged and resuspended in fresh media without hydroxynorvaline but containing 25 µg/ml of cycloheximide to stop further protein synthesis and the incubation continued. At 30-min intervals, 1-ml aliquots were removed, centrifuged, and the resuspended cells and media treated with sodium dodecyl sulfate and mercaptoethanol as described in Fig. 2. Zero time in the table is after 30 min of incubation with the [14C]proline.

Experiment 1—Hydroxynorvaline present during synthesis period but absent during secretory period. Fibroblasts were incubated with or without 1 mM hydroxynorvaline for 20 min and then [14C]proline was added to a final concentration of 2 µCi/ml and the incubation continued for 30 min. At that time the cells were centrifuged and resuspended in fresh media without hydroxynorvaline but containing 25 µg/ml of cycloheximide to stop further protein synthesis and the incubation continued. At 30-min intervals, 1-ml aliquots were removed, centrifuged, and the resuspended cells and media treated with sodium dodecyl sulfate and mercaptoethanol as described in Fig. 2. Zero time in the table is after 30 min of incubation with the [14C]proline.

Experiment 2—Hydroxynorvaline absent during synthesis period but present during secretory period. Fibroblasts were incubated with 10^{-4} M colcemide for 20 min and then [14C]proline was added to a final concentration of 2 µCi/ml and the incubation continued for 30 min. The colcemide causes intracellular retention of hydroxylated procollagen (32). Cycloheximide was added to a final concentration of 25 µg/ml to both the control and experimental flasks and hydroxynorvaline to 1 mM to the experimental flask and the incubation continued for 15 min. The cell suspensions were centrifuged at 1500 × g for 2 min and the control cells resuspended in fresh media without colcemide but containing 25 µg/ml of cycloheximide and the experimental cells resuspended in media without colcemide but containing 25 µg/ml of cycloheximide and 1 mM hydroxynorvaline. At the times indicated, 1-ml aliquots were removed and treated as in Experiment 1 above.

| Time (min) | Control 14C CPM | Hydroxynorvaline 14C CPM |
|------------|-----------------|-------------------------|
| 0          | 14.0            | 9.5                     |
| 30         | 13.6            | 8.6                     |
| 60         | 13.8            | 9.1                     |
| 90         | 14.4            | 8.5                     |

TABLE IV

| Time (min) | Control 14C CPM | Hydroxynorvaline 14C CPM |
|------------|-----------------|-------------------------|
| 0          | 14.1            | 13.2                    |
| 30         | 14.5            | 15.0                    |
| 60         | 13.2            | 12.7                    |
| 90         | 13.6            | 14.4                    |

* Hydroxynorvaline present during synthesis but absent during secretion.


discussion

Hydroxynorvaline was shown by Buston et al. (35) to specifically antagonize the effects of threonine in stimulating the growth of S. faecalis but did not antagonize the effect of threonine. Since labeled hydroxynorvaline was not available and since the chemical amounts of protein synthesized by the tendon cells during the incubations was extremely small, it was not possible for us to demonstrate directly the incorporation of hydroxynorvaline into protein. However, there is little doubt that it specifically replaced threonine in being transferred to tRNA (Table II), and that it markedly inhibited the incorporation of [3H]threonine into protein synthesized by the tendon cells (Fig. 2). Once amino acid analogues have been transferred to tRNA there is no known further physiological process which will prevent their incorporation into protein. Although we can not rigorously exclude other unknown effects of the analogue, it is likely that hydroxynorvaline effectively replaced threonine in the synthesized procollagen.

Although the mechanism of secretion of collagen has been studied for a number of years in different types of cells, many
selectively bind to membrane-bound ribosomes. All proteins secreting cells distinguish which proteins are to be secreted and which are to be retained intracellularly have not been elucidated. Two general hypotheses can be formulated: (a) mRNA coding for secreted proteins contains a tag which allows it to selectively bind to membrane-bound ribosomes. All proteins synthesized on membrane-bound ribosomes are vectorially discharged through the membrane into the cisternae. (b) Endoplasmic reticular membranes selectively recognize and bind proteins which are to be secreted and they are transferred into the cisternae. In both mechanisms, proteins transported into the cisternae are then secreted, possibly passing through other cell organelles or compartments.

In the present work we have found that incorporation of an analogue specific for threonine interferes with the secretion of procollagen. This inhibition of secretion was not caused by interference with the hydroxylation of proline resulting in destabilization of the triple helical portion of the molecule since the molecules containing the analogue were hydroxylated to the same extent as the control. However, approximately 50% of the substituted procollagen chains found intracellularly were not disulfide-linked, a much higher fraction than that found in the control synthesized in the presence of colcemide. It is likely, but not conclusively established, that it is this fraction which was not perfectly triple helical and hence sensitive to pepsin at all temperatures in the range 15–37°C (Table V). The remaining 50% of the substituted procollagen chains found intracellularly were vectorially discharged on membrane-bound ribosomes and are vectorially discharged through the membrane into the cisternae.

![Fig. 4. Agarose gel chromatography of procollagen containing hydroxynorvaline after incubation with and without pepsin. Fibroblasts (10^7/ml) were incubated for 2 hours with 1 μCi/ml of [¹³C]proline and 1 mM hydroxynorvaline. The cell suspensions were centrifuged at 1,500 x g for 2 min and the cells resuspended in 2 ml of 0.6 M acetic acid. One-milliliter aliquots were then incubated with or without 100 μg/ml of pepsin for 6 hours at 15°C. At the end of the digestion the samples were then prepared for chromatography as described under "Experimental Procedures." Aliquots were chromatographed at room temperature on a column (1.5 x 80 cm) of 6% agarose (Bio-Gel A-5m, 200 to 400 mesh, Bio-Rad Laboratories). The fraction size was 2.0 ml. Marker β rat tail collagen eluted at Fraction 26 and α rat tail collagen at Fraction 32. Unsubstituted, normal procollagen (not shown) eluted at Fraction 30. O...O, Incubation without pepsin; •...•, with pepsin.]

| Digestion temperature | [¹³C]Procollagen | Pepsin resistant |
|-----------------------|-----------------|-----------------|
| 15°C                  | 3.3             | 56              |
| 20                    | 3.4             | 59              |
| 25                    | 3.6             | 54              |
| 30                    | 3.4             | 50              |
| 35                    | 3.5             | 58              |
| 37                    | 3.6             | 57              |
Fig. 5. Polyacrylamide disc gel electrophoresis of intracellular procollagen prepared for electrophoresis in the presence or absence of reducing agent. Aliquots of cells which had been incubated for 2 hours with [14C]proline and with 1 mM hydroxynorvaline (HNV) or 1 μM colcemide (control) were resuspended in 0.01 M sodium phosphate, pH 7.4, and 1% sodium dodecyl sulfate containing either 1% mercaptoethanol or 0.1 M iodoacetamide. Electrophoresis and counting of the gels was then carried out as described under “Experimental Procedures.” ○–○. Prepared for electrophoresis with 1% mercaptoethanol; ◦–◦, prepared with 0.1 M iodoacetamide. Under the conditions of electrophoresis, the disulfide-linked procollagen barely entered the gel and was largely lost from the top of the gel during the workup and slicing procedure.

nondisulfide-linked ones may form two separate overlapping sets rather than a single set and molecules in either set may not be secreted normally. The concept that correct conformation and assembly of procollagen chains is essential for normal secretion is supported by the observation that the molecules synthesized in the presence of hydroxynorvaline but which were nevertheless secreted were comparable to control procollagen as measured by disulfide linkage and pepsin sensitivity (Fig. 6). The conformational requirement could be involved in either a recognition step as discussed above or in a packaging mechanism.

Continued incubation of the fibroblasts with hydroxynorvaline resulted in progressive inhibition of protein synthesis as measured by a general decrease in incorporation of all labeled amino acids. The accumulation of the substituted procollagen may have caused this inhibition either through a specific feedback mechanism or through nonspecific blockage. Alternatively, a protein which is itself necessary for continued protein synthesis may be defective when it contains hydroxynorvaline, and accumulation of this defective factor may lead to inhibition of further protein synthesis.

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