Neonatal rat lung prolyl hydroxylase was synthesized in a cell-free system. Total polysomes were isolated from neonatal rat lung homogenates after Triton X-100 and deoxycholate solubilization. The polysomes were then translated in a wheat germ lysate in the presence of tritiated leucine. The radioactive product was precipitated with antibody to rat prolyl hydroxylase and co-precipitated with protein A (Pansorbin). The synthesis of rat lung prolyl hydroxylase was optimal at 4.6 mM magnesium and 135 mM potassium chloride. Spermine (16.6 mM) was also present in the cell-free system. On non-denaturing gels, 61% of the labeled protein which was precipitated with antibody alone co-migrated with tetrameric prolyl hydroxylase. The radioactive precipitated product was also analyzed on sodium dodecyl sulfate-polyacrylamide gels after denaturation with urea and reduction with mercaptoethanol. On denaturing gels, 64% of the radioactive product migrated coincidently with the subunits of the mature rat skin enzyme.

Total neonatal rat lung polysomes contain prolyl hydroxylase activity which was inhibited by antibody. The prolyl hydroxylase-containing polysomes were isolated from total lung polysomes by immunoprecipitation. The antibody-precipitated polysomes did not synthesize collagen. However, 44% of the total radioactive leucine-labeled product migrated coincidently with the purified enzyme subunits on denaturing gels. Therefore, neonatal rat lung polysomes are able to direct the synthesis of prolyl hydroxylase in the wheat germ lysate system. These data indicate that newly synthesized lung prolyl hydroxylase is composed of two newly synthesized and distinct subunits which are of the same apparent size on sodium dodecyl sulfate gels as the α and β subunits of the mature enzyme.

Collagen, the major connective tissue protein of mammals, differs from other proteins both in its unusual amino acid composition and the post translational steps of its biosynthesis. Collagen contains trans-4-hydroxyproline which is not derived from free proline but is derived from the hydroxylation of certain proline residues of collagen nascent chains attached to polysomes of the rough endoplasmic reticulum (for a review, see Ref. 1).

Prolyl hydroxylase (EC 1.14.11.4), an intracisternal enzyme (2-5), is the mixed-function oxygenase which catalyzes the post translational hydroxylation of proline residues in collagen nascent chains to form hydroxyproline (for a review, see Ref. 6). The enzyme requires ascorbate, molecular oxygen, ferrous ion, and α-ketoglutarate for activity (7). During the hydroxylation of proline in collagen nascent chains, oxygen is simultaneously incorporated into α-ketoglutarate to form succinate and carbon dioxide (8). Ascorbate serves as the probable reducing agent.

Prolyl hydroxylase has been purified to homogeneity from newborn rat skin (9) by conventional chromatographic techniques, and from chick embryos (10) and human fetal skin (11) by affinity chromatography. The purified enzyme is a tetramer containing two nonidentical subunits with molecular weights of 60,000 and 64,000 (12, 13). The monomers have a half-life of 37.9 h (14). The tetramer has been reported to have a half-life of 39 h (14), while another study demonstrated a half-life of 73 h (15).

An inactive protein which cross-reacts with antibody to purified prolyl hydroxylase was originally found to exist in cultured fibroblasts (18). A subsequent study demonstrated that various mammalian tissues contain this cross-reactive protein (17). A recent study demonstrated homology between the cross-reacting protein and the smaller subunit of the tetrameric enzyme (12).

A role of the immunologically cross-reacting protein in the synthesis of tetrameric prolyl hydroxylase in cell culture has been proposed (18, 26). Pulse-chase experiments indicated that both the larger α subunit of the tetrameric enzyme and the cross-reacting protein were labeled. However, the smaller β subunit of the tetrameric enzyme was not labeled until 24 h later. These studies in cell culture demonstrate that tetrameric prolyl hydroxylase is assembled from newly synthesized α chains and from preformed β subunits. In the present report, polysomes were isolated from neonatal rat lung and run off in
the wheat germ lysate system. The data indicate that both subunits of prolyl hydroxylase are synthesized simultaneously on neonatal rat lung polysomes, and that prolyl hydroxylase-synthesizing polysomes contain active enzyme which synthesizes the tyrosine aminotransferase in the ribosomal runoff system containing both α and β newly synthesized subunits. The data furthermore indicate that although prolyl hydroxylase is an intracisternal microsomal protein, the initial products of translation are not synthesized as precursor forms which are detectable by SDS1 gel electrophoresis.

**EXPERIMENTAL PROCEDURES**

**Materials**—One- to three-day-old Sprague-Dawley rats obtained from Canadian Breeding Labs (St. Constant, Canada) were used for the preparation of lung polysomes and purified rat skin prolyl hydroxylase. All chemicals used in these studies were analytical reagent grade. L-[3,4,5-3H]Leucine (150 Ci/mmol), Aquasol-2, and Triton X-100 were obtained from New England Nuclear Corp. (Boston, MA). [5-3H]Proline (25 Ci/mmol) was obtained from Amersham (Arlington Heights, IL). Form III bacterial collagenase was obtained from Advanced Biofactures Corp. (Lynbrook, NY). The wheat germ was kindly supplied by Mr. Malhotra of General Mills (Minneapolis, MN). RNase-free sucrose was purchased from Schwarz/Mann Co. (Orangeburg, NY). Pansorbin (protein A) was obtained from Calbiochem Corp. (La Jolla, CA). Acrylamide, N,N'-methylenebisacrylamide, N,N',N'-tetramethylethylenediamine, and bromophenol blue were obtained from Eastman Kodak Co. (Rochester, NY). Poly-L-proline, type II (M, = 1000 to 9000), and Coomassie brilliant blue R were purchased from Sigma Chemical Co. (St. Louis, MO).

**Purification of Prolyl Hydroxylase**—Prolyl hydroxylase was purified from neonatal rat skin and chicken embryos with the following modifications of two affinity column chromatography methods described previously (10, 20, 21). Neonatal rat skin (1600 g) was homogenized in 1600 ml of 0.1 M Tris-HCl (pH 7.8) containing 0.2 M NaCl, and 0.1 M glycine (enzyme buffer) at 4°C. The homogenization was carried out using a Polytron ST homogenizer (Brinkmann Co., Westbury, NY) at 4°C speed. The homogenate was then centrifuged at 12,000 × g for 45 min and the pellet was discarded. The enzyme was precipitated with ammonium sulfate, passed through an agarose affinity column containing reduced and carboxymethylated Ascaris cuticle collagen, and then separated from poly-L-proline, which was used as eluant by Sephadex G-100. The purified enzyme was precipitated with ammonium sulfate and dialyzed against 100 mM potassium chloride. The sample was diluted with 100 mM potassium chloride, 10% sucrose, and 0.5 M Tris-HCl (pH 7.5) were added. The sample was then homogenized in a motor-driven glass Teflon homogenizer with seven strokes. The homogenate was centrifuged at 9000 g for 10 min. The supernatant was adjusted through glass wool, then made 0.5% with sodium deoxycholate, and 0.25% with Triton X-100, and homogenized with five hand strokes in a glass Teflon homogenizer. The sample was layered over 7 ml of a 2 M sucrose solution containing the original homogenizing buffer, and was centrifuged at 130,000 × g for 5 h. The supernatant was washed with 0.25 M sucrose, 5 mM MgCl₂, 0.1 mM EDTA, 1 mM dithiothreitol (pH 7.0) (ribosomal suspension media). The polysomal solution was cleared by centrifuging at 12,000 × g for 10 min. The resulting supernatant was aliquoted and stored at −80°C until assayed.

**Immunoprecipitation of Polysomes**—Polysomes (154 A₂₆₀ units A₂₆₀ = 50 g of RNA) suspended in the ribosomal suspension medium were combined with 1 ml of antiserum and incubated for 48 h at 4°C. The sample was centrifuged at 20,000 × g for 60 min. The remaining polysomal pellet was suspended in the ribosomal suspension medium, aliquoted, and stored at −80°C until assayed. Heparin was not added to the polysomal precipitation buffer, since we wished to translate the antibody-precipitated polysomes. Heparin was found to inhibit protein synthesis directed by polysomes.

**Preparation of the Wheat Germ Extract**—The 30,000 × g supernatant of wheat germ homogenate was prepared as described previously (23).

**Prolyl Hydroxylase Activity**—Prolyl hydroxylase activity of total lung polysomes was determined by the tritium release assay (7). Triton X-100 was not added to the incubation mixture, since addition of this detergent partially inactivated polysome-bound prolyl hydroxylase.

**Protein Synthesis in Vitro**—The protein-synthetic system contained 40 μg of protein of the 30S wheat germ extract, 0.6 mg adenosine triphosphate, 16.7 μM guanosine triphosphate, 6.7 μM creatine phosphate, 33.3 gm/g of creatine phosphokinase, 135 mM potassium chloride, 4.6 mM magnesium acetate, 0.033 μM of 19 amino acids minus leucine, 10 μCi of [4,5-3H]leucine, 16.6 μM spermine, and 20 mM (2-imidazoyl)-pyrazinecarboxylic acid (Hepes) (pH 7.5) made up to a final volume of 60 μl with H₂O. The reaction mixture also contained either 0.5 A₂₆₀ unit of total lung polysomes or 0.44 A₂₆₀ unit of immunoprecipitated lung polysomes. The reaction mixture was incubated for 1 h at 25°C.

**Collagenase Digestion of the Radioactive Product**—Total lung polysomes and immunoprecipitated lung polysomes were incubated in the wheat germ extract containing [5-3H]proline. The products formed were digested with purified collagenase.

**Immunoprecipitation of Radioactive Prolyl Hydroxylase**—The protein-synthetic reaction mixture was made 2% (w/v) with sodium deoxycholate, 0.1% (w/v) Triton X-100, and 50 μM with leucine. Antiserum (20 μl) was added, and the solution was mixed and allowed to incubate at room temperature for 1 h. Pansorbin (11 mg) was added, and the sample was blended on a Vortex mixer and then allowed to incubate at room temperature for 1 h. Two and one-half volumes of a buffer containing 2% (w/v) Triton X-100, 2% (w/v) sodium deoxycholate, 200 mM NaCl, 20 mM ethylenediaminetetraacetic acid, and 50 mM Tris-HCl (pH 7.1) were added. The sample was blended on a Vortex mixer and then centrifuged at 30,000 × g for 20 min at 4°C. The supernatant was aspirated, and the resulting pellet was resuspended in 1 ml of 0.5 N NaOH and incubated for 45 min at 37°C with periodic mixing. One and two-tenths milliliters of 0.5 M HCl was added, the sample was blended on Vortex mixer, and 0.5 ml of this sample was counted in Aquasol-2.

**Denaturing Disc Gel Electrophoresis of the Synthesized Product**—Immunoprecipitated protein was precipitated with antibody and not Pansorbin and washed thoroughly. The total product of immunoprecipitated polysomes was suspended in running buffer consisting of 1% (w/v) dibasic sodium phosphate, 0.4% (w/v) monobasic sodium phosphate, 0.1% SDS, 0.1% Triton X-100, and 50 mM with leucine. The sample was then washed in water, lyophilized, and suspended in running buffer. Both samples were then made 3 M urea and 1% (w/v) SDS, and incubated for 0.5 h at 37°C. The sample was then made 5% (v/v) in mercaptoethanol and incubated at 37°C for 0.5 h. Cylindrical disc gels (0.5 x 12 cm) containing 7.7% acrylamide, 0.15% (w/v) ammonium persulfate, 0.1% SDS, 1% (w/v) sodium dodecylsulfate, and 0.4% monobasic sodium phosphate. N,N'-Methylenebisacrylamide and N,N,N',N'-tetramethylethylendiamine were added for cross-linking. An aliquot of each sample was made 0.08% with bromphenol blue and electrophoresed. The resulting dyes was 1 cm from the bottom of the gel. The gels were then rinsed in water and stained in a 0.05% Coomassie blue solution of methanol/ acetic acid (5:1, v/v) for 2 h. The gels were destained in 7% (v/v) acetic acid and 5% (v/v) methanol for 48 h. Other gels were frozen and cut into 1-mm slices. Each gel was then placed in a glass vial containing 2% (v/v) hydrogen peroxide and 25% ammonium hydroxide. The samples were then acidified with concentrated acetic acid and counted in Aquasol-2.

**Nondenaturing Acrylamide Gel Electrophoresis**—Cylindrical disc gels (10.5 x 12 cm) were used consisting of 7.7% (w/v) acrylamide, 0.07% (w/v) ammonium persulfate, and 25 mM Tris/glycine (pH 8.3). N,N'-Methylene bisacrylamide and N,N,N'-tetramethylethylendiamine were added for cross-linking. The immunoprecipitated protein was suspended in buffer consisting of 50 mM Tris/glycine (pH 8.3) and electrophoresed at 4°C at 12 mA/gel. The gels were cut into 1-mm slices, digested, and prepared for counting as described above.

**RESULTS**

Polysomes were isolated from the lungs of neonatal rats. A
Newly Synthesized Prolyl Hydroxylase

The sucrose gradient profile of these polysomes appears in Fig. 1. The presence of large intact polysomes is indicated. The polysomes were next translated in the wheat germ lysate cell system in the presence of tritiated leucine. The product formed was incubated with antiserum to purified rat skin prolyl hydroxylase and co-precipitated with Pansorbin (protein A). Maximum precipitation was observed with 10 to 50 µl of antiserum (data not shown). In all subsequent assays, 20 µl of antiserum were used. The amount of radioactive leucine incorporated into antibody-precipitated prolyl hydroxylase represents 6% of total amount of leucine incorporated into protein.

The amount of radioactive precipitate was also dependent on the amount of Pansorbin added as co-precipitant. Maximum precipitation of radioactive product occurred with 10 µl of Pansorbin (0.16 mg). In subsequent experiments, 11 mg of Pansorbin were used as co-precipitant. Both 0.16 and 11 mg of Pansorbin precipitated the same amount of radioactive product.

Both the magnesium and potassium chloride concentrations in the cell-free protein-synthesizing system were optimized. Synthesis of prolyl hydroxylase was maximal at a concentration of 4.6 mM MgCl₂ (data not shown). A potassium chloride concentration of 155 mM was required for optimal synthesis of radioactive prolyl hydroxylase.

The amount of product synthesized in the wheat germ lysate was dependent on the quantity of polysomes added (Fig. 2). Linearity of radioactive product synthesized occurred with up to 0.25 A₂₆₂₀ units of polysomes added to the lysate system. The addition of more polysomes resulted in a nonlinear increase of radioactive product synthesized.

Total lung polysomes contain prolyl hydroxylase activity (Fig. 3). This enzyme activity was lost when the polysomes were assayed in the presence of antibody to purified rat enzyme. Nonionic detergents added to the polysome during enzyme assay partially inactivated enzyme (data not shown). Polysomal prolyl hydroxylase activity does not represent incomplete subunit activity. The subunits of prolyl hydroxylase are unable to hydroxylate chick embryo substrate (24, 25). Furthermore, the polysomes are not just contaminated with cisternal prolyl hydroxylase. Antibodies to purified rat skin prolyl hydroxylase precipitate polysomes which do not synthesize collagen but do synthesize distinct proteins which migrate coincident with the subunits of the tetrameric enzyme (Fig. 5B).

Tetrameric prolyl hydroxylase was labeled when polysomes were translated in the wheat germ lysate (Fig. 4). Total lung polysomes were translated in the presence of [³H]leucine. The product was precipitated with antiserum to purified prolyl hydroxylase. The antigen-antibody complex was then washed extensively and chromatographed on a non-denaturing acrylamide gel (Fig. 1).

![Fig. 1. Profile of total lung polysomes. Four A₂₆₂₀ units of polysomes suspended in 0.25 M sucrose (RNase-free), 1 mM dithiothreitol, 0.1 mM ethylenediaminetetraacetic acid, and 5 mM MgCl₂ were layered over 30 ml of a 10 to 30% (w/v) linear sucrose gradient. The sucrose solution contained 10 mM Tris-HCl (pH 7.5), 1 mM dithiothreitol, and 5 mM MgCl₂. The gradient was centrifuged at 90,000 x g for 2 h and was then fractionated and monitored at A₂₆₂₀.](image)

![Fig. 2. Titration of the ³H-labeled immunoprecipitate versus the amount of polysomes used. Each reaction mixture contained the components described in the text and varying amounts of total lung polysomes. The product was precipitated with antibody and co-precipitated with Pansorbin as described in the text.](image)

![Fig. 3. Prolyl hydroxylase activity of total lung polysomes. Polysomes were isolated as described in the text and assayed for prolyl hydroxylase activity by the tritium release assay. The open circles indicate the prolyl hydroxylase activity of polysomes assayed in the presence of 20 µl of antiserum against purified rat enzyme.](image)

![Fig. 4. The migration on a non-denaturing acrylamide gel of leucine-labeled product made by total lung polysomes and precipitated with prolyl hydroxylase antiserum. Leucine-labeled product synthesized by wheat germ lysate was precipitated with antiserum as described. The precipitated material was washed six times and electrophoresed on a 7.7% Tris/glycine-acrylamide gel (pH 8.3). Of the 10,365 dpm applied to the gel, 6,235 were in the tetrameric enzyme band. The arrow indicates the migration of tetrameric rat and chick embryo prolyl hydroxylase.](image)
amidgel. Sixty-one per cent of the leucine-labeled product migrated coincidently with tetrameric rat skin and chick embryo prolyl hydroxylases. Duplicate gels were run and stained with Coomassie blue. The antibody separated from the radioactive enzyme on nondenaturing gels.

The molecular weight of the antibody-precipitated radioactive product was also analyzed on SDS-acrylamide gels after denaturation (Fig. 5A). As indicated in Fig. 5A, greater than 60% of the antibody-precipitated product migrated coincidently with the subunits of purified rat skin enzyme. Radioactive products also migrated as lower molecular weight chains which are probably incomplete subunit peptides, since radioactive peptides of molecular weights greater than \( \alpha \) and \( \beta \) subunits are not seen on the SDS gels. However, larger molecular weight peptides are seen in the gel of the leucine-labeled products of total lung polysomes (Fig. 6). Also, note that the large peak of small molecular weight peptides beyond 70 mm is absent when the antibody-precipitated material is analyzed on a denaturing gel (Fig. 5A).

Prolyl hydroxylase-synthesizing polysomes were isolated by immunoprecipitation. Total polysomes were incubated with antiserum, and the precipitated polysomes were isolated by centrifugation. The isolated polysomes were translated in the wheat germ lysate and the total synthetic product was analyzed on SDS-acrylamide gels. Forty-four per cent of the radioactive enzyme on nondenaturing gels. Sixty-one per cent of the leucine-labeled product migrated coincidently with the subunits of purified rat skin enzyme. Radioactive products also migrated as lower molecular weight chains which are probably incomplete subunit peptides, since radioactive peptides of molecular weights greater than \( \alpha \) and \( \beta \) subunits are not seen on the SDS gels. However, larger molecular weight peptides are seen in the gel of the leucine-labeled products of total lung polysomes (Fig. 6). Also, note that the large peak of small molecular weight peptides beyond 70 mm is absent when the antibody-precipitated material is analyzed on a denaturing gel (Fig. 5A).

The molecular weight of the subunits of both purified rat skin and chick embryo prolyl hydroxylases used as standards were determined on denaturing gels (Fig. 7). The molecular weight of the small subunit was 60,000, while that of the larger subunit was 65,000. These values agree within experimental error to the values of 60,000 and 64,000 reported in the literature (12, 13).

Active enzyme is on prolyl hydroxylase-synthesizing polysomes and not collagen-synthesizing polysomes. The total product of the antiserum-precipitated polysomes migrated as purified subunits and nascent chains (Fig. 5B). Furthermore, while the total lung polysomes synthesized proline-labeled

![Fig. 5.](image5.jpg)

**Fig. 5.** The migration of the leucine-labeled immunoprecipitate from total lung polysomes and the product made from antiserum-precipitated lung polysomes. A, chromatogram of a gel containing the immunoprecipitate from total lung polysomes. The immunoprecipitate was dissolved in running buffer, dialyzed extensively against \( \mathrm{H}_2\mathrm{O} \), lyophilized, and resuspended in running buffer. The sample was denatured, reduced, electrophoresed, and prepared for counting as described under "Experimental Procedures." Of a total of 22,510 dpm applied to the gel, 16,335 were in the subunit bands. The arrows indicate the migration of the subunits (\( \alpha \), \( \beta \), and \( \gamma \)) of rat skin prolyl hydroxylase. B, chromatogram of product made by the antibody-precipitated lung polysomes. The product was denatured, reduced, and prepared for electrophoresis as described for A. Nineteen times the amount of radioactive product as in A was applied to determine whether any radioactive product larger than the subunits of prolyl hydroxylase was present, thus assuring the purity of the antiserum-precipitated polysomes. Of a total of 482,500 dpm applied to the gel, 212,150 were in the subunit bands.

![Fig. 6.](image6.jpg)

**Fig. 6.** Polyacrylamide gel analysis of the total leucine-labeled product synthesized by lung polysomes in the wheat germ lysate system. The total leucine-labeled product of the wheat system was deacylated by making the reaction mixture 1.5 M in \( \mathrm{Tris} \) (pH 10.0) and incubating at 37°C for 20 min. The reaction mixture was dialyzed extensively against \( \mathrm{H}_2\mathrm{O} \), lyophilized, and resuspended in running buffer. The sample was then denatured, reduced, electrophoresed, and prepared for counting as described under "Experimental Procedures." The total leucine-labeled product applied to the gel (648,000 dpm) represents and one-half the number reaction mixtures used for the experiment shown in Fig. 4, and two-thirds the number of reaction mixtures used for the denaturing gel (Fig. 5A).

![Fig. 7.](image7.jpg)

**Fig. 7.** Migration of protein standards and rat skin and chick embryo subunits of prolyl hydroxylase on denaturing gels. Purified protein standards and rat skin and chick embryo enzymes were electrophoresed on SDS-polyacrylamide gels as described in the text. The molecular weights of the \( \alpha \) and \( \beta \) subunits of both chick embryo and rat skin enzyme were 65,000 and 60,000, respectively.
TABLE I
Comparison of collagen synthesis by total lung and antisera-precipitated polysomes

<table>
<thead>
<tr>
<th>Polysome preparation</th>
<th>Radioactivity remaining after collagenase digestion</th>
<th>Digestion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-Collagenase-</td>
<td>+Collagenase</td>
</tr>
<tr>
<td>Total lung polysomes</td>
<td>16.9</td>
<td>10.7</td>
</tr>
<tr>
<td>Precipitated polysomes</td>
<td>23.7</td>
<td>23.0</td>
</tr>
</tbody>
</table>

TABLE II
Purification of prolyl hydroxylase-synthesizing polysomes by antibody precipitation

<table>
<thead>
<tr>
<th>Polysomes</th>
<th>dpm as subunits/ A260 unit of polysome</th>
<th>Purification fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>Antibody-precipitated</td>
<td>20.3</td>
<td>71.8</td>
</tr>
</tbody>
</table>

A product which was 37% digestible by purified bacterial collag- enase, only 3% of the product directed by the antibody- precipitated polysomes was digested (Table I). The potassium and magnesium concentrations in the cell-free system were optimal for collagen synthesis (data not shown). This 3% digestion of the proline-labeled product of the antibody-precipitated polysomes was negligible, since this level of digestion was also obtained with exogenous [3-H]tryptophan-labeled Escherichia coli protein (data not shown). The antibody- precipitated polysomes are as active and more so in incorporating proline into protein as the total lung polysomes. The polysomes were therefore not inactivated by degradation during antibody precipitation.

By comparing the amount of radioactive subunits of enzyme synthesized per A260 unit of polysomes, we have determined the degree of purification of prolyl hydroxylase-synthesizing polysomes by antibody precipitation (Table II). Greater than a 71-fold purification was obtained by immunoprecipitation.

DISCUSSION

Polysomes were isolated from neonatal rat lung and translated in a cell-free lysate system prepared from wheat germ extract. Greater than 60% of the immunoprecipitated radioactive product was identified as a product migrating coincidentally with tetrarmeric prolyl hydroxylase from rat skin on non-denaturing gels, and coincidentally with the enzyme subunits on denaturing gels.

Cell-free translational systems which measure the ability of polysomal and mRNA to synthesize a specific protein are useful. Pulse-labeling experiments which measure the ability of tissues and cells to synthesize specific proteins after drug administration and in physiological and pathological states are complicated by possible changes in precursor pool specific activity and the preferential use of specific isoaccepting tRNA species for the synthesis of certain proteins. If the decrease of protein synthesis results from a decrease of functioning mRNA, the use of polysomal and mRNA-dependent cell-free systems obviates these problems.

This report indicates that tetrameric prolyl hydroxyxylase is synthesized on polysomes. Polysomes contain prolyl hydroxylase activity which can be inactivated by antibody. Furthermore, the immunoprecipitated polysomes direct the synthesis of enzyme but not collagen in vitro. The antiserum-precipitated product migrated coincidentally with tetrameric enzyme on non-denaturing gels. On denaturing gels, both of the subunits were radioactive. Although other studies (18, 26) have demonstrated that in cultured chick embryo tendon fibroblasts, only the larger subunit was synthesized during a pulse period, the evidence presented here indicates that freshly isolated rat lung polysomes can direct the synthesis of the tetrameric protein in which both subunits are labeled.

Prolyl hydroxylase has been shown to be localized within the cisternae of the rough endoplasmic reticulum by ferritin-labeled antibody (2) and subcellular fractionation studies (3-5). In addition, underhydroxylated nascent collagen peptides are localized within the cisternae of the rough endoplasmic reticulum (4).

Many secretory proteins and some microsomal membrane proteins are synthesized as precursor forms containing an NH2-terminal hydrophobic extension which is removed by a membrane-associated peptidase (27, 28). Our data indicate that although prolyl hydroxylase is an intracisternal microsomal protein (2-4), its subunits are not synthesized as precursor forms which are significantly larger, as determined on SDS gels, than the subunits of the mature active enzyme. The “signal” NH2-terminal extension may actually remain a component of the mature membrane-associated proteins, as has been recently shown for phenobarbital-induced cytochrome P-450 (29).

REFERENCES

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19. Deleted in proof