Effects of Procollagen Peptides on the Translation of Type II Collagen Messenger Ribonucleic Acid and on Collagen Biosynthesis in Chondrocytes†

Larry M. Paglia,* Manfred Wiestner,‡ Michael Duchene,‡ Lucille A. Ouellette, Dietrich Hörlein,‡ George R. Martin,§ and Peter K. Müller‡

ABSTRACT: Type II procollagen messenger ribonucleic acid (mRNA) was isolated from chick sternum and rat chondrosarcoma cells and translated in a reticulocyte lysate cell-free system. A high molecular weight band was identified as type II procollagen by gel electrophoresis, collagenase digestion, and specific immunoprecipitation. The translation of type II mRNA was specifically inhibited by addition of type I procollagen amino-terminal extension peptide. When this peptide was added to the media of cultured fetal calf chondrocytes, chick sternal chondrocytes, or chick tendon fibroblasts, no inhibition of collagen synthesis was evident. These data suggest a general regulation of collagen biosynthesis by these peptides in the cell-free translation system. However, as indicated by the cell culture experiments, cellular characteristics and evolutionary divergence of animal species seem to restrict the effect of the peptides.

Collagen biosynthesis varies quantitatively during development, in response to trauma, and in certain disease states (Prockop et al., 1980). In the case of collagen types I–IV, the component chains are synthesized as large precursors, the pro α chains, each translated from a distinct messenger ribonucleic acid (mRNA). The original translation products include short signal peptides which are removed shortly after formation as has been reported for other proteins destined for secretion (Palmiter et al., 1979). The procollagen chains are modified posttranslationally by the glycosylation and hydroxylation of certain amino acid residues. They are then assembled into a triple-helical procollagen molecule, and during or after secretion specific proteases remove peptides from the amino and carboxy termini of the chains. The C-terminal peptide probably determines the solubility of the protein since removal of these peptides is associated with precipitation of the proteins into fibers.

The regulation of collagen synthesis is not well understood. Some studies suggest that certain enzymes modifying the pro α chains regulate the rate of collagen production (Cardinale & Udenfriend, 1974; Ristelli & Kivirikko, 1976; Siegel et al., 1978). Other studies indicate that the rate of collagen synthesis is proportional to and controlled by the amount of specific pro α chain mRNA's (Adams et al., 1977; Moen et al., 1979).

Recently, it has been reported that the N-terminal extension peptides from types I and III procollagen appear to specifically reduce collagen synthesis when added to the media of cultured fibroblasts (Krieg et al., 1978; Wiestner et al., 1979). In addition, we have shown that these peptides inhibit the translation of type I procollagen mRNA in a reticulocyte lysate cell-free system (Paglia et al., 1979). In this study, we have extended our investigation of the effects of type I and type III N-terminal peptides to the synthesis of cartilage type II collagen by chondrocytes and to the translation of proα1(II) mRNA in the reticulocyte lysate cell-free system.

Materials and Methods

Formula 947 scintillation fluid, [^{3}H]proline, and translation-grade [^{35}S]methionine were obtained from New England Nuclear, [^{14}C]proline was from Amersham Searle, purified bacterial collagenase, type III, was from Advanced Biofactures, Pansorbin was from Calbiochem, oligo(dT)–cellulose, T-3, was from Collaborative Research, and all other enzymes were from Boehringer-Mannheim. Tissue culture media (Dulbecco's minimum Eagle's medium, Ham's F12), Falcon plastic ware, trypsin (2.5%), fetal calf serum, streptomycin, and clostridial collagenase (EC 3.4.24.3, Worthington, CLS) were supplied by Laborservice, Munich. Penicillin was from Chemie Grünenthal GmbH, Stolberg i. Rheintal, Federal Republic of Germany.

RNA Isolation. RNA was extracted from day 15 chick embryo sterna and from a rat chondrosarcoma tumor. The tissue was dissected free of perichondrium and capsular material, respectively, and extracted immediately in 8 M guanidine-HCl as previously described (Strohman et al., 1977; Adams et al., 1977). Total RNA isolated in this manner was chromatographed on oligo(dT)–cellulose to further purify poly(A+) mRNA (Breitkreutz et al., 1978). Where indicated, this mRNA was fractionated by centrifugation at 200000*g* for 5–6 h on 5–20% linear sucrose gradients (Rosen et al., 1975) in a Beckman SW41 rotor. RNA was also isolated from the calvaria of 1-day-old rats as previously described (Paglia et al., 1979) and used as a template for the synthesis of type I procollagen.

Cell-Free Synthesis. Reticulocyte lysate was prepared as described by Rowe et al. (1978) with the exception that the column chromatography step was eliminated. Endogenous translation activity was reduced by treatment with staphylococcal nuclease (Pelham & Jackson, 1976). Translation reactions were performed in 15-μL aliquots containing 5 μL of lysate, RNA as indicated, 1.75 mM MgOAc, 140 mM KOAc, 40 mM KCl, 20 mM *N*-2-(hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid (Hepes), pH 7.4, 15 mM creatine phosphate, 50 μg/mL creatine kinase, 100 μM each of 19 unlabeled amino acids, and one radioactive amino acid, either [^{35}S]-methionine, [^{3}H]proline, or [^{14}C]proline. Reactions were in-

† From the Departments of Biochemistry and Medicine, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20014. *Received November 21, 1980.*

‡ Present address: Max-Planck-Institute of Biochemistry, Department of Connective Tissue Research, D-8033 Martinsried bei Munich, Federal Republic of Germany.

§ Present address: Laboratory of Developmental Biology and Anomalies, National Institute of Dental Research, National Institutes of Health, Bethesda, MD 20205.

cubated for 90 min at 30 °C, stopped by the addition of 5 μg of pancreatic ribonuclease, and further incubated for 15 min. A 5-μL aliquot was removed from each reaction, decolorized, and trichloroacetic acid (Cl_3CCOOH) precipitated, the insoluble material was collected on glass fiber filters, and radioactivity was determined by scintillation counting as previously described (Paglia et al., 1979).

Immunoprecipitation. Antibody to rat type II collagen, isolated from chondrosarcoma tumor tissue, was produced in rabbits and purified by affinity chromatography on a type II collagen–Sepharose column (Becker et al., 1976). This IgG preparation did not cross-react with types I or III procollagen, and no species specificity was evident. Addition of types I or III amino-terminal extension peptides to the immunoprecipitation had no noticeable effect at the concentrations used in these experiments. Precipitation of type II procollagen was performed by addition of 15 μL of the translation reaction to 125 μL of 50 mM phosphate buffer, pH 7.4, 100 mM NaCl, 1 mM EDTA, 0.1% bovine serum albumin, 1 mM unlabeled proline or methionine, 0.5% deoxycholate, 0.5% Triton X-100, and 20 μg of specific IgG. Samples were incubated for 1 h at 37 °C, and then 200 μL of Pansorbin, a 10% suspension of killed *Staphylococcus aureus* cells with coat protein A, was added. This suspension was incubated at 4 °C for 30 min and then centrifuged at 10000*g* for 5 min. The supernatant fluid was aspirated and the pellet washed with up to 1 mL of 10 mM Tris, pH 8.0, 150 mM NaCl, and 0.5% Triton X-100. The suspension was centrifuged and the pellet washed twice more. Immunoprecipitated material was released from the Pansorbin by suspension of the pellet in 50 μL of 10 mM Tris, pH 8.1, 5 mM dithiothreitol, and 2% sodium dodecyl sulfate ($NaDodSO_4$) and heating at 100 °C for 5 min. Samples were immediately centrifuged at 10000*g* for 5 min, and the supernatant fluid was removed either for scintillation counting or for gel electrophoresis.

Amino-Terminal Peptide Addition. The isolation and characterization of amino-terminal extension peptides have already been described (Wiestner et al., 1979). Either type I, col-1(I), or type III, col-1(III), peptide was dissolved in 20 mM Hepes, pH 7.4, and added to the translation mixture at the start of the reaction in the indicated amounts.

Gel Electrophoresis. Samples for electrophoresis were suspended in 62.5 mM Tris, pH 6.8, 0.5 M urea, 2% $NaDodSO_4$, 10% glycerol, and 12 μg/mL bromophenol blue and heated to 90 °C for 5 min. Dithiothreitol was added to a final concentration of 0.2 mM, and samples were layered on 5–7% acrylamide slab gels (Laemmli, 1970). Following electrophoresis, the gels were prepared for fluorography (Bonner & Laskey, 1974) and dried, and radioactive bands were visualized by exposure of X-ray plates.

Type II collagen and procollagen used as markers for electrophoresis were radiolabeled and isolated as described by Uitto (1977) with the exception that whole, day 15, chick sterna were used rather than isolated matrix-free cells.

Collagenase Digestion. For specific digestion of cell-free product, duplicate 15-μL reaction mixtures were brought up to 45 μL and a final concentration of 17 mM calcium acetate and 6 mM *N*-ethylmaleimide. Samples received either bacterial collagenase (5 units) in 25 mM Tris, pH 7.4, and 0.33 M calcium acetate or the buffer without enzyme as a control. Reactions were incubated at 37 °C for up to 180 min and stopped by the addition of gel sample buffer.

Cell Cultures. Chondrocytes were isolated from articular cartilage of fetal calves and from sternal cartilage of 17-day-old chick embryos according to established methods (Dehm & Prockop, 1973). Chick tendon fibroblasts were obtained as described by Dehm & Prockop (1971).

Falcon dishes (60 mm) were inoculated with 5×10^6 cells. Cells were counted with a hemocytometer and tested for viability by trypan blue exclusion. Chondrocytes and fibroblasts were maintained in monolayers with Ham's F12 and Dulbecco's minimum Eagle's media, respectively. Both culture media contained 10% fetal calf serum and the usual ingredients as described eariler (Müller et al., 1977).

For our experiments, we used chondrocytes after 3 days in culture (synthesizing type II collagen) and also after 5 weeks in culture (synthesizing mainly type I collagen). The latter cells were passaged 6 times with 0.25% trypsin–0.05% EDTA solution and were split in a 1:2 ratio. Tendon fibroblasts were used after 3 days in culture for the investigations. Dedifferentiation of the chondrocytes was recorded by morphological observations, and the switch of collagen synthesis from type II to predominant type I collagen was analyzed with CM-cellulose chromatography and polyacrylamide gel electrophoresis of the isolated collagen (Müller et al., 1977; Benya et al., 1978).

Radioactive Labeling of Collagen and Quantitation of Collagen Synthesis in Cultured Cells. Cells were preincubated for 24 h with the culture medium lacking streptomycin, after which the medium was replaced by 2 mL of the incubation medium which consisted of Dulbecco's minimum Eagle's medium without serum, penicillin (400 units/mL), sodium ascorbate (50 μg/mL), β-aminopropionitrile fumarate (50 μg/mL), and L-[2,3-^{3}H]proline (5 μCi/mL). The incubation was carried out for 24 h at 37 °C. Col-1(I) was added to the respective samples at a concentration of 6 μM. The measurement of protein-bound [^{3}H]hydroxyproline and [^{3}H]proline in the medium and cell layer has recently been described (Wiestner et al., 1979). Briefly, cell suspensions and media were pooled, heated to 60 °C for 1 h, and dialyzed against 0.5% acetic acid, then 1 M $CaCl_2$ and 0.05 M Tris, pH 7.4, and finally against 0.5% acetic acid. The protein was lyophilized and hydrolyzed in 6 N HCl, with mercaptoethanol, at 110 °C for 24 h under nitrogen. The hydrolysate was evaporated, redissolved in 0.1 M sodium citrate buffer, pH 2.2, and filtered on Beckman-type M82 ion-exchange resin. Separation of [^{3}H]hydroxyproline and [^{3}H]proline was performed on a Beckman Multichrom amino acid autoanalyzer. The values obtained for radioactively labeled hydroxyproline and proline were used to calculate the percent of collagen with respect to total protein. The amount of collagen as a percentage of total protein was determined with the formula:

$$\% \text{ collagen} = \frac{2(\text{cpm Hyp})}{5(\text{cpm Pro} - \text{cpm Hyp}) + 2(\text{cpm Hyp})} \times 100$$

This is based on the assumption that collagen contains approximately equal amounts of proline (Pro) and hydroxyproline (Hyp) and about 5 times as many imino acids as noncollagenous proteins (Diegelmann & Peterkofsky, 1972).

Results

RNA Translation. In preliminary experiments, it was noted that RNA extracted from chick sternum or rat chondrosarcoma was poorly translated in the reticulocyte lysate cell-free system. This was in contrast to the translation of RNA from type I procollagen synthesizing tissues. Following chromatography on an oligo(dT)–cellulose column to eliminate the poly(A–) fraction, translation of mRNA was enhanced both in terms of total incorporation and in the synthesis of high molecular weight proteins. The products of cell-free translation

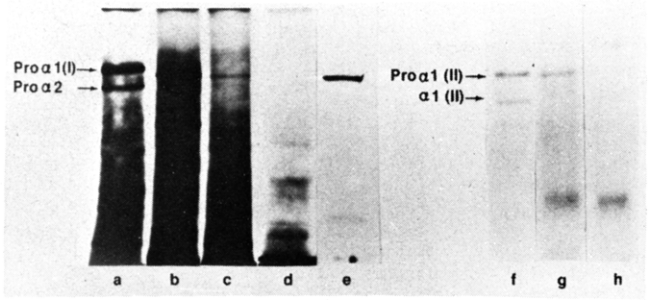

FIGURE 1: Gel electrophoresis of cell-free translation products; tracks a–e are labeled with [^{35}S]methionine and f–h with [^{14}C]proline. Track a, translation with 0.1 μg of rat calvaria mRNA. Arrows indicate proα1(I) and proα2(I). Tracks b and c, translation with 0.1 μg of chick sternum mRNA and rat chondrosarcoma mRNA, respectively. Track d, endogenous reticulocyte lysate incorporation. Track e, immunoprecipitation of chick sternum mRNA translation product with type II collagen antibodies. Track f, marker type II collagenous proteins isolated from cultured chick sterna. Arrows indicate type II procollagen (upper) and collagen bands. Tracks g and h, translation of chick sternum mRNA followed by incubation without (g) and with (h) collagenase.

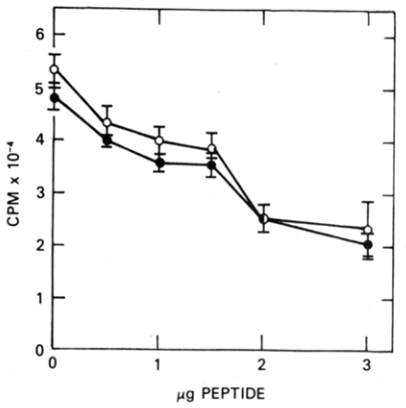

FIGURE 2: Cl_3CCOOH-precipitable radioactivity from cell-free translations with increasing amounts of type I amino-terminal extension peptide. (○) Translation with 0.1 μg of rat calvaria mRNA; (●) with 0.1 μg of chick sternum mRNA. [^{3}H]Proline precursor was used in these reactions, and 1 μg of peptide represents a final concentration of approximately 4 μM.

by using sternum and chondrosarcoma mRNA labeled with [^{35}S]methionine and [^{14}C]proline are shown in Figure 1. A large component of approximately 150 000 daltons is evident. This band migrates with marker proα1(II) upon electrophoresis, is collagenase sensitive, and is specifically immunoprecipitated with anti type II collagen IgG's. This component migrates between proα1(I) and proα2(I) and is not precipitated by antiserum against type I procollagen or against proteoglycan core protein (not shown).

Comparing the relative intensity of the putative proα1(II) bands from sternum and chondrosarcoma, it is evident that the sternum provides more proα(II) template activity than an equivalent amount of chondrosarcoma mRNA.

Additional evidence for the identity of the proα1(II) band was provided by an examination of the size of the mRNA coding for it. The mRNA coding for type I procollagen has been shown to sediment in the 28S region of a sucrose density gradient (Boedtker et al., 1976; Breitkreutz et al., 1978). Preliminary data on rat chondrosarcoma mRNA suggested a similar 28S size for type II procollagen messenger (Diaz de Leon et al., 1980). This result was confirmed by centrifuging sternum mRNA on sucrose gradients and pooling fractions corresponding approximately to 0–15, 15–26, 26–30, and 30–35 S. When the RNA from each fraction was translated, a band corresponding to putative proα1(II) was evident in the 26–30S fraction (not shown). A similar band, but of reduced intensity, was synthesized by the 30–35S fraction, suggesting that this procollagen mRNA either sediments to the "heavy" side of the 28S region or is not sharply resolved into a discrete peak by this gradient.

Efficient translation of type II procollagen mRNA was more difficult than translation of type I procollagen mRNA. The latter could be translated by use of unfractionated RNA even at high concentrations, although the synthesis of proα1(I) and proα2(I) chains was no longer in a 2:1 ratio (Paglia et al., 1979). The isolated mRNA from normal chondrocytes translated in a similar fashion; however, the mRNA from the rat chondrosarcoma cells exhibited a very sharp optimum range for synthesis. The range of concentrations in which this mRNA was translated was limited (0.5–0.15 μg/reaction), and at higher concentrations the proα1(II) band visible following electrophoresis and fluorography was dramatically reduced (not shown). However, this sharp optimum was no longer observed when the 26–30S region of the chondrosarcoma mRNA was used for translation. This indicates that a component of the chondrosarcoma inhibits translation but can be separated from the proα1(II) mRNA by centrifugation.

Inhibition of Type II Procollagen mRNA Translation by Amino-Terminal Extension Peptide. The amino-terminal extension peptides from both types I and III procollagen have been shown to inhibit the translation of type I procollagen mRNA in the reticulocyte lysate (Paglia et al., 1979). Maximal inhibition was observed at a peptide concentration of 4–6 μM. In this study, increasing amounts of col-1(I) peptide were added to cell-free reactions with either rat calvaria or chick sternum mRNA present in a constant amount. In both cases, the amount of Cl_3CCOOH-precipitable incorporation was reduced with [^{3}H]proline as the radiolabeled precursor (Figure 2). The degree of inhibition was identical for both preparations of mRNA, with an approximate reduction of 30% at 1 μg of added peptide and 50% decrease with 2 μg.

Increasing amounts of either col-1(I) or col-1(III) peptide were added to a translation reaction with chick sternum mRNA. Subsequently, the [^{3}H]proline-labeled proteins were incubated with specific antibodies to type II collagen to precipitate reacting materials. Using this assay, it was found that col-1(I) peptide reduced type II procollagen synthesis by more than 60% while col-1(III), the type III peptide, caused little or no inhibition (Figure 3). The col-1(III) peptide also had no significant effect on the total incorporation of labeled amino acids into the Cl_3CCOOH precipitate (data not shown).

Similar studies were carried out with [^{35}S]methionine as the labeled precursor, where sternal RNA was translated in the presence and absence of col-1(I) peptide. The use of [^{35}S]-methionine in contrast to [^{3}H]proline emphasizes non-collagenous polypeptides formed since collagenous proteins have a relatively lower methionine content. Under these conditions, a general inhibition of protein synthesis was not apparent since total Cl_3CCOOH-precipitable radioactivity remained constant even at relatively high (12 μM) peptide concentrations. The cell-free reaction produces were separated by electrophoresis as shown in Figure 4. The formation of proα1(II) was markedly reduced while the synthesis of other components was essentially unchanged.

Effect of N-Terminal Peptide [Col-1(I)] on Cultured Cells. Previous studies have shown that the col-1(I) peptide from the proα1(I) chain of type I procollagen inhibited collagen synthesis when added to cultures of human and calf skin fibroblasts. In contrast, it was observed that the synthesis of collagen (type II) by calf chondrocytes was not inhibited.

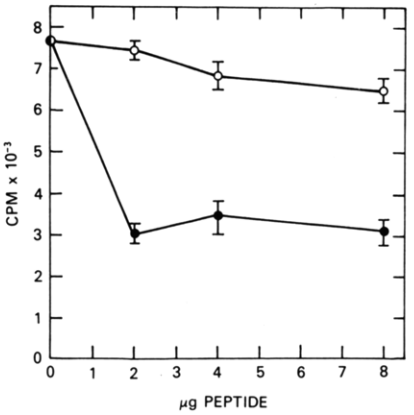

FIGURE 3: Immunoprecipitation of chick sternum mRNA translation with increasing concentrations of col-1(I) (●) and col-1(III) (○) peptides. Reactions contained 0.1 μg of mRNA and [^{3}H]proline radiolabeled precursor.

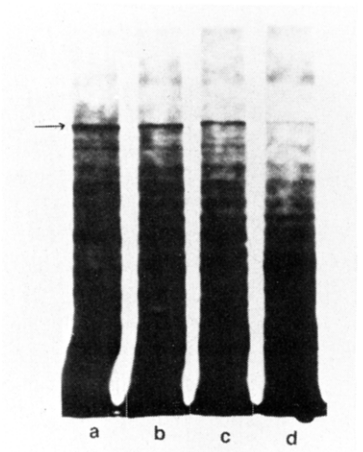

FIGURE 4: Gel electrophoresis of chick sternum mRNA translation with added col-1(I) peptide. All reactions contained 0.1 μg of mRNA and [^{35}S]methionine precursor. Track a, no added peptide; track b, 0.5 μg of peptide; track c, 1 μg of peptide; track d, 2 μg of peptide.

Similar experiments were carried out as part of this study. Collagen synthesis by cultured calf chondrocytes exhibits variability depending on the age of the donor. Since the exact age of the calves used was not available, cells were isolated and cultured from two animals of distinctly different ages. When col-1(I) was added to chondrocyte cultures from either source, no inhibition of collagen synthesis was noted (Table I). Further, chondrocytes which had been subcultured until they assumed a "fibroblastic" morphology and synthesized type I rather than type II collagen were not affected by added col-1(I) (Table I).

Since col-1(I) inhibited the translation of type II collagen mRNA from chick rather than calf tissue, similar experiments were carried out with cultured chick chondrocytes. Again, col-1(I) did not significantly alter collagen synthesis in these chondrocytes (Table II). In dedifferentiated fibroblastic cells derived from these chondrocytes, collagen synthesis was likewise unaffected. However, the col-1(I) peptide also had no effect on collagen production by fibroblasts cultured from chick tendon (Table II). As found earlier, this peptide significantly inhibited collagen synthesis when added to cultures of calf skin fibroblasts (not shown).

Table I: Effect of Col-1(I) on Collagen Synthesis by Calf Chondrocytes

chondrocytes	cells/cm^2 (X10^5)	% collagen of total protein	
		controls	+ col-1(I)
synthesizing type II collagen	1.77	19.4 ± 0.9 (6)[a]	18.2 ± 1.0 (6)
	1.56	5.8 ± 0.4 (8)	5.9 ± 0.3 (7)
synthesizing type I collagen	1.77	8.4 ± 1.0 (6)	7.0 ± 0.8 (6)
	0.52	3.5 ± 0.3 (6)	3.7 ± 0.4 (5)

[a] Number of measurements in parentheses.

Table II: Effect of Col-1(I) on Collagen Synthesis by Cultured Chick Cells

chondrocytes	% collagen of total protein	
	controls	+ col-1(I)
synthesizing type II collagen	12.1 (1)[a]	13.9 (7)
synthesizing mainly type I collagen	12.2 ± 0.9 (6)	11.2 ± 1.1 (6)
tendon fibroblasts	5.3 ± 2.7 (4)	6.2 ± 2.9 (4)

[a] Number of measurements in parentheses.

Discussion

Recently, it has been observed that the addition of peptides derived from the N-terminal regions of proα1(I) and proα1-(III) chains to cultured human and calf fibroblasts reduces the rate of collagen synthesis without altering the synthesis of other proteins (Krieg et al., 1978; Wiestner et al., 1979). These observations supported the results of earlier studies indicating an increase in collagen synthesis in cells with decreased conversion of procollagen to collagen, specifically cells from patients with Ehlers-Danlos VII syndrome (Lichtenstein et al., 1973) and animals with a related genetic defect, dermatosparaxis (Lenaers et al., 1971). These peptides have also been found to persist in the media of certain cultured cells (Pontz et al., 1973), bovine amniotic fluid (Nowack et al., 1976), and in serum from fetal calves (Rohde et al., 1976) and humans with chronic liver disease (Rohde et al., 1976), all instances of elevated collagen production.

In addition, it has been noted that peptides prepared from the N-terminal region of proα1(I) and proα1(III) chains inhibit the translation of mRNA for proα1(I) and proα2(I) in a reticulocyte lysate cell-free system (Paglia et al., 1979). Col-1(I), a peptide released from procollagen by treatment with collagenase, including residues 1–99 of the proα1(I) chain, was active in reducing procollagen mRNA translation. Reduction and alkylation of col-1(I) largely destroyed its inhibitory activity, indicating that the conformation of this peptide was critical. While the translation of mRNA's for proα1(I) and proα2(I) was inhibited, little effect was noted on the translation of mRNA's for globin, vittelogenin, and other noncollagenous proteins present in the type I mRNA preparation.

Taken together, the data are consistent with the involvement of this peptide in the regulation of collagen synthesis by feedback inhibition. There is an obvious need for such a mechanism since, following synthesis, collagen is deposited extracellularly in insoluble fibers. The data on the effects of the peptide in the reticulocyte lysate system suggest that regulation of translation in calf and human fibroblasts is the site of action, although this has not been proven.

The purpose of this study was to investigate the specificity of N-terminal peptide inhibition with respect to type II collagen synthesis by chondrocytes and cell-free translation of type II procollagen mRNA. Previous studies have shown that the mRNA for proα1(II) can be translated in vitro, giving rise to a polypeptide which migrates electrophoretically between proα1(I) and proα2(I) (Cheah et al., 1979; Upholt et al., 1979; Diaz de Leon et al., 1980). We have obtained similar results in our studies. The identification of the putative proα1(II) chain was based on collagenase susceptibility and its precip-

itation by type II collagen specific antibodies. Isolation of poly(A+) RNA from total RNA by passage over oligo-(dT)–cellulose significantly improved the translation of proα1(II) mRNA as did further purification by sucrose density sedimentation. In the latter case, the mRNA appeared to sediment on the "heavy" side of 28S RNA. Cartilage contains very high amounts of proteoglycans with a large proportion of sulfated carbohydrate side chains. These may be carried along during the RNA isolation and could possibly interfere with translation, thus necessitating further purification of cartilage mRNA. Since chondrosarcoma mRNA was particularly sensitive to the possible presence of an inhibitor and had a very sharp concentration optimum for translation, the chick sternum mRNA was used for the peptide studies.

Added col-1(I) peptide inhibited the translation of mRNA for proα1(II) at levels previously shown to inhibit the translation of type I procollagen mRNA from rat calvaria (Paglia et al., 1979). In contrast, col-1(III) was not inhibitory although it was shown previously to suppress the translation of proα(I) and proα2(I) mRNA. While the N-terminal peptides from the proα(I) and proα1(III) chains are similar, they are also chemically and immunologically distinct. Our previous study showed that these peptides did not significantly reduce the synthesis of other proteins in the translation reaction at concentrations that significantly reduced procollagen synthesis. When the type II procollagen mRNA was translated with [^{35}S]methionine precursor, the resulting electrophoretic profile revealed a significant reduction in the proα1(II) band while many lower molecular weight bands retained their intensity.

Addition of col-1(I) to cultured chondrocytes from calf or chick did not alter collagen synthesis nor did the peptide affect chondrocytes which had been allowed to dedifferentiate by continued culture and which synthesize primarily type I collagen. Further, the col-1(I) peptide did not inhibit the synthesis of collagen by chick tendon fibroblasts. Such observations suggest that there are additional factors in cells not present in the lysate which determine the activity of such peptides. One possibility would be receptors or a transport system necessary for the passage of peptides into the cell. Such factors could increase the specificity of peptide action with respect to collagen types and particular tissues.

While the mechanism of N-terminal peptide inhibition remains unclear, our data support earlier work suggesting a role for these peptides in the regulation of collagen synthesis.

References

Adams, S. L., Sobel, M. E., Howard, B. H., Olden, K., Yamada, K. M., deCrombrugge, B., & Pastan, I. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 3399.

Becker, U., Nowack, H., Gay, S., & Timpl, R. (1976) *Immunology 31*, 57.

Benya, P. B., Padilla, S. R., & Nimni, M. E. (1978) *Cell (Cambridge, Mass.) 15*, 1813.

Boedtker, H., Frischauf, A. M., & Lehrach, H. (1976) *Biochemistry 15*, 4765.

Bonner, W. M., & Laskey, R. A. (1974) *Eur. J. Biochem. 46*, 83.

Breitkreutz, D., Diaz de Leon, L., Paglia, L., Zeichner, M., Wilczek, J., & Stern, R. (1978) *Biochim. Biophys. Acta 517*, 349.

Cardinale, G. J., & Udenfriend, S. (1974) *Adv. Enzymol. Relat. Areas Mol. Biol. 41*, 245.

Cheah, K. S. E., Grant, M. E., & Jackson, D. S. (1979) *Biochem. Biophys. Res. Commun. 91*, 1025.

Dehm, P., & Prockop, D. J. (1971) *Biochim. Biophys. Acta 240*, 358.

Dehm, P., & Prockop, D. J. (1973) *Eur. J. Biochem. 35*, 159.

Diaz de Leon, L., Paglia, L., Breitkreutz, D., & Stern, R. (1977) *Biochem. Biophys. Res. Commun. 77*, 11.

Diaz de Leon, L., Breitkreutz, D., Zeichner, M., Stern, R., & Paglia, L. (1980) *Connect. Tissue Res. 7*, 135.

Diegelmann, R. F., & Peterkofsky, B. (1972) *Dev. Biol. 28*, 443.

Krieg, T., Hörlein, D., Wiestner, M., & Müller, P. K. (1978) *Arch. Dermatol. Res. 263*, 171.

Laemmli, U. K. (1970) *Nature (London) 277*, 680.

Lenaers, A., Ansay, M., Nusgens, B., & Lapiere, C. M. (1971) *Eur. J. Biochem. 23*, 533.

Lichtenstein, J. R., Martin, G. R., Kohn, L. D., Byers, P. H., & McKusick, V. A. (1973) *Science (Washington, D.C.) 182*, 298.

Moen, R. C., Rowe, D. W., & Palmiter, R. D. (1979) *J. Biol. Chem. 254*, 3526.

Müller, P. K., Lemmen, C., Gay, S., Gauss, V., & Kühn, K. (1977) *Exp. Cell Res. 108*, 47.

Nowack, H., Rohde, H., & Timpl, R. (1976) *Hoppe-Seyler's Z. Physiol. Chem. 357*, 601.

Paglia, L. M., Wilczek, J., Diaz de Leon, L., Martin, G. R., Hörlein, D., & Müller, P. K. (1979) *Biochemistry 18*, 5030.

Palmiter, R. D., Davidson, J. M., Gagnon, J., Rowe, D. W., & Bornstein, P. (1979) *J. Biol. Chem. 254*, 1433.

Pelham, H. R., & Jackson, R. J. (1976) *Eur. J. Biochem. 67*, 247.

Pontz, B. F., Müller, P. K., & Meigel, W. N. (1973) *J. Biol. Chem. 248*, 7558.

Prockop, D. J., Kivirikko, K. I., Tuderman, K., & Guzman, N. A. (1980) *N. Engl. J. Med. 301*, 77.

Ristelli, J., & Kivirikko, K. I. (1976) *Biochem. J. 158*, 361.

Rohde, H., Nowack, H., Becker, U., & Timpl, R. (1976) *J. Immunol. Methods 11*, 135.

Rosen, J. M., Woo, S. L. C., & Comstock, J. P. (1975) *Biochemistry 14*, 2895.

Rowe, D. W., Moen, R. C., Davidson, J. M., Byers, P. H., Bornstein, P., & Palmiter, R. D. (1978) *Biochemistry 17*, 1581.

Siegel, R. C., Chen, K. H., Greenspan, J. S., & Aguiar, J. M. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 2945.

Strohman, R. C., Moss, P. S., Micov-Eastwood, J., Spector, D., Przybyla, A., & Paterson, B. (1977) *Cell (Cambridge, Mass.) 10*, 265.

Uitto, J. (1977) *Biochemistry 16*, 3421.

Upholt, W., Vertel, B. M., & Dorfman, A. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 4847.

Wiestner, M., Krieg, T., Hörlein, D., Glanville, R. W., Fietzek, P., & Müller, P. K. (1979) *J. Biol. Chem. 254*, 7016.