Inhibition of Procollagen Cell-Free Synthesis by Amino-Terminal Extension Peptides†

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ABSTRACT: Peptides prepared from the amino termini of pro α1(I) and pro α1(III) collagen chains inhibit the production of pro α1(I) and pro α2 by rat calvaria RNA in a reticulocyte cell-free system. The synthesis of other proteins was not altered, suggesting a specific effect on collagen production. Various peptides from the helical region of the α1(I) chain did not alter translation. These studies, taken together with earlier studies showing inhibition of collagen synthesis by cells in culture receiving the amino-terminal peptides, are consistent with a regulatory function in collagen synthesis for the amino-terminal peptides from procollagen.

Changes in the type and amount of collagen present in a tissue are associated with embryonic development and many disease states, indicating that these two parameters are normally closely regulated. Presumably, the type of collagen in the tissue is determined by the selection of the appropriate genes for transciption. Additionally, the cells synthesizing collagen must have some mechanism for determining the amount of collagen present in the tissue and reducing production when the appropriate tissue level is attained.

Regulation of collagen production could be manifest at transcription, at translation, or at some subsequent step prior to secretion. Considerable attention has been directed toward possible regulation by enzymes that modify the peptide chains of the protein. For example, specific modifications of the peptide include the enzymatic hydroxylation of certain of the prolyl and lysyl residues to form peptide-bound hydroxyproline and hydroxylysine. Decreases in the formation of hydroxyproline are known to reduce the stability and secretion of collagen (Berg & Prockop, 1973; Jimenez et al., 1973). Changes in the rate of such posttranslational modifications could, therefore, regulate the amount of collagen deposited in the extracellular matrix.

Recent studies suggest that peptides removed from the NH_2-terminal end of procollagen during its conversion to collagen are able to inhibit collagen synthesis (Krieg et al., 1978; Wiestner et al., 1979). These amino-terminal peptides are removed en bloc from pro α chains by a specific protease (Lapiere et al., 1971). These peptides are large (~10000 M_r) and have been detected in a variety of biological fluids (Pontz et al., 1973; Nowack et al., 1976; Rohde et al., 1976). Recently, Wiestner et al. (1979) have found that the addition of the NH_2-terminal peptides prepared from a collagenase digest of either the pro α1(I) chain or type III procollagen inhibits collagen synthesis by cultured bovine and human skin fibroblasts. Concentrations of 4–6 μM peptide are required to achieve 50% inhibition. No change in the synthesis of other proteins was observed at these concentrations.

Here we report studies concerning the effect of NH_2-terminal peptides on the synthesis of procollagen in a reticulocyte lysate cell-free system. The translation of mRNA coding for type I procollagen as well as for several other noncollagenous proteins was tested. The results indicate a specific inhibition of procollagen synthesis by these NH_2-terminal peptides.

Materials and Methods

Translation-grade [^{35}S]methionine was obtained from New England Nuclear, purified bacterial collagenase, type III, was from Advanced Biofactures, goat antirabbit immunoglobulins were from Miles, and Hydromix scintillation fluid was from Yorktown Research.

Peptides. The NH_2-terminal peptides from types I and III procollagen were prepared as previously described (Furthmayr et al., 1972; Becker et al., 1976a). Briefly, type I P_n-collagen[1] was extracted from dermatosparactic calf skin with neutral salt in the presence of protease inhibitors. The extracted protein was digested with bacterial collagenase, and the amino-terminal peptide [col 1(I)] was purified by column chromatography first on DEAE-cellulose, then on agarose 1.5 M, and finally on phosphocellulose. Type III peptide [col 1(III)] was derived from cyanogen bromide treatment of type III procollagen extracted from fetal calf skin. The cyanogen bromide peptides were separated by column chromatgoraphy on carboxymethylcellulose, and col 1(III) was further purified by collagenase digestion, followed by gel filtration. Col 1(I) contains five disulfide bridges and has a molecular weight of 10700. The conformation, size, and relative location in the NH_2 terminus of Col 1(I) are shown in Figure 1. Col 1(III), with five intrachain and one interchain disulfide bridges, has a molecular weight of 45000, and each of its three identical chains resembles col 1(I) in amino acid composition.

Three chemically modified derivatives of col 1(I) were also tested. Col 1(I)RC, prepared by reduction and carboxymethylation, retained the same number of amino acids as the original peptide (98). Following trypsin treatment, another sample was designated col 1(I)T. This peptide retained 88 amino acids and had a molecular weight of 9400. A third sample was treated with staphylococcal protease which cleaves portions of the NH_2 and COOH terminals, leaving a peptide, col 1(I)SP, with 68 amino acids (M_r 6500). The relative size and segments lost by enzymatic cleavage of the latter two

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[1] P_n-collagen, procollagen following cleavage of the carboxy-terminal extension peptide; Cl_3AcOH, trichloroacetic acid; EDTA, ethylenediaminetetraacetic acid; $NaDodSO_4$, sodium dodecyl sulfate; CNBr, cyanogen bromide; CB3, -6, -7, and -8, peptides derived from cyanogen bromide treatment of α1(I) collagen.

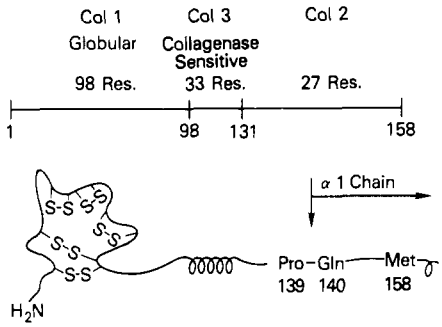

FIGURE 1: Amino-terminal peptide from pro α1(I). This represents the entire 158 amino acid residues, including the col 1(I) segment used in these experiments, that are normally cleaved after helix formation. This is from Rohde et al. (1976).

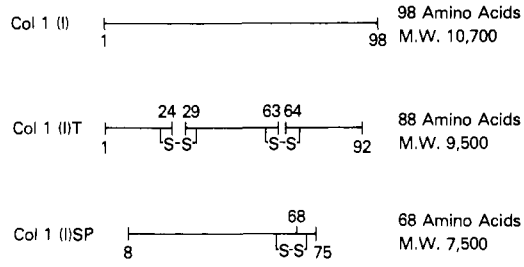

FIGURE 2: Comparison of col 1(I) and derived peptides following treatment with either trypsin [col 1(I)T] or staphylococcal protease [col 1(I)SP].

peptides are depicted in Figure 2.

Cyanogen bromide peptides from rat α1(I) chains were made according to Bornstein & Piez (1966).

RNA. RNA was extracted from cells or total tissue by using 6 M guanidine hydrochloride according to the method of Strohman et al. (1977). Calvaria were dissected from newborn rats and placed directly into guanidine for extraction. Globin RNA was extracted from rabbit reticulocytes, and vitellogenin RNA, a gift of Dr. William H. Kastern, National Cancer Institute, was isolated from the liver of roosters treated with estrogen. No further purification of the RNA was done, and all preparations stimulated protein synthesis when added to the translation system.

Cell-Free Synthesis. Reticulocyte lysate was prepared as described by Rowe et al. (1978) and was treated with staphylococcal nuclease to reduce endogenous translational activity (Pelham & Jackson, 1976). Translation was performed in 125-μL aliquots containing 50 μL of lysate, RNA as indicated under Results, 1 mM ATP, 0.2 mM GTP, 1.75 mM $Mg(OAc)_2$, 140 mM KOAc, 40 mM KCl, 0.3 mM spermidine, 20 mM Hepes, pH 7.6, 15 mM creatine phosphate, 50 μg/mL creatine kinase, and 100 μM 19 unlabeled amino acids and 1 radioactive amino acid. Either [^{35}S]methionine, [^{3}H]proline, or [^{14}C]proline was used in the reaction. Reactions were incubated for 3 h at 26 °C, after which 1/10 volume was removed, decolorized with H_2O_2 and NaOH (Pelham & Jackson, 1976), and precipitated with 8% Cl_3AcOH, 0.5% tannic acid, and 2 mM proline or methionine. Cl_3AcOH-insoluble material was collected on glass fiber filters, washed with 5% Cl_3AcOH and counted in 0.5 mL of water and 10 mL of Hydromix. The remaining reaction was terminated by adding either 1/10 volume of Stop Mix (5% Triton X-100, 5% deoxycholate, and 4 mM proline or methionine) for immunoprecipitation or an equal volume of twice-concentrated gel sample buffer (125 mM Tris-HCl, pH 6.8, 1 M urea, 4% $NaDodSO_4$, 20% glycerol, and 24 μg/mL bromophenol blue) used for gel electrophoresis. When collagenase digestion was to be performed, the reaction was first stopped by incubation at 37 °C for 20 min with ribonuclease (200 μg/mL). Then 20 units of bacterial collagenase was added, the reaction was adjusted to 17 mM Ca^{2+} and 6 mM *N*-ethylmaleimide, and incubation was continued for 90 min at 37 °C.

Immunoprecipitation. Antiserum to rat type I procollagen was produced in rabbits and purified by affinity chromatography on a type I procollagen–Sepharose affinity column (Becker et al., 1976b). Purified IgG's were used for specific precipitation of cell-free product at a concentration of 100 μg/mL and were incubated for 1 h at room temperature. This concentration of IgG was far in excess of the amount necessary for maximal precipitation of synthesized procollagen. Even at very high concentrations, however, some collagenase-sensitive material remains in the supernatant fraction. This is due, most likely, to the presence of incompleted, partially denatured, or otherwise nonantigenic collagenous peptides synthesized in the cell-free reaction. This concentration of IgG proved sufficient for maximal precipitation of cell-free product even at the highest levels of added type I or type III peptide. Purified goat antirabbit IgG was then added and allowed to react overnight at 4 °C. The immune precipitate was isolated by centrifugation at 10000*g* for 10 min at 4 °C, through sucrose cushions of 0.5 M and 1.0 M sucrose in 0.5% Triton X-100 and 10 mM EDTA. The supernatant fraction was aspirated down to the sucrose, the tube was washed with water, and the fraction was finally aspirated down to the pellet. The precipitate isolated in this manner retained little or no free labeled amino acid. For scintillation counting, pellets were dissolved in 0.4 mL of 0.2 N acetic acid and counted in 10 mL of Hydromix.

Gel Electrophoresis and Densitometry. Samples for electrophoresis were run on 5–7% acrylamide slab gels (Laemmli, 1970), and the radioactive bands were visualized by using fluorography (Bonner & Laskey, 1974). [^{14}C]-Procollagen standards were made from 3T6 cells and purified by salt precipitation and DEAE-cellulose column chromatography (Smith et al., 1972). Following fluorography and development of the X-ray plate, bands were scanned at 525 nm by using a densitometer which plotted the curves and integrated the area beneath each one. This procedure was done routinely to establish the proportion of pro α1 and pro α2 in the translation products.

Results

Translation of Calvaria RNA. As shown by others (Rowe et al., 1978; Monson & Goodman, 1978), the reticulocyte lysate cell-free system, pretreated with *Staphylococcus aureus* nuclease, translates the procollagen messengers in calvaria RNA into full-sized pro α1(I) and pro α2 chains. We use a higher K^+ level than others (180 mM vs. 90 mM) and incubate for 3 h rather than for 90 min at 26 °C. These modifications enhance the formation of the pro α chains. For example, while total protein synthesis increases little after 90 min, there is a 40–60% increase in the protein precipitated by specific antiserum to type I procollagen (data not shown). The higher level of K^+ has a similar effect of enhancing procollagen rather than total protein synthesis. The additional K^+ is derived from potassium acetate rather than KCl since a concentration of chloride ion higher than 40 mM is inhibitory to cell-free translation (Weber et al., 1977).

The patterns of [^{35}S]methionine and [^{14}C]proline proteins obtained by the translation of calvaria RNA are shown in Figure 3. Among the polypeptides observed upon gel electrophoresis are two which migrate in the same region as authentic pro α chain standards. Only these two peptides are

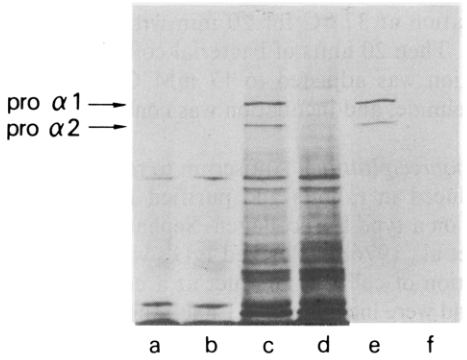

FIGURE 3: Gel electrophoresis of rat calvaria RNA cell-free product. Tracks a–d were proteins labeled with [^{35}S]methionine; e and f were proteins labeled with [^{14}C]proline. Track a: endogenous translational activity of the lysate without added calvaria RNA. Track b: endogenous cell-free product digested with bacterial collagenase. Track c: translation with 1 μg of rat calvaria RNA. Track d: rat calvaria product digested with bacterial collagenase. Track e: same as track c, with [^{14}C]proline. Track f: [^{14}C]proline-labeled product treated with bacterial collagenase. Procollagen markers (not shown) were prepared as described under Materials and Methods and run with each gel.

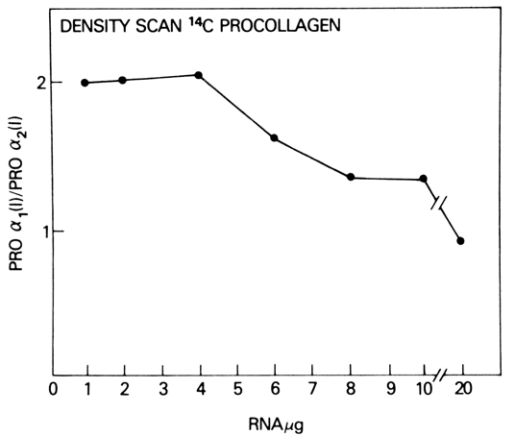

FIGURE 4: Effect of increasing RNA concentration on cell-free synthesis of procollagen. All reactions were 125 μL, and, following gel electrophoresis and fluorography, the pro α1 and pro α2 bands were scanned with a densitometer to give the relative ratio of protein in the two bands.

removed by prior incubation with collagenase and can be precipitated by purified antibody to type I procollagen (data not shown). As expected from its amino acid composition, these two collagenase-sensitive bands are the two major labeled products when proline is used as the radioactive precursor (Figure 3, track e). We conclude that the two bands observed are pro α1(I) and pro α2.

To assure translational fidelity in the cell-free reaction, we determined the ratio of pro α1(I) to pro α2 synthesized as described under Materials and Methods. At lower concentrations of calvaria RNA (0.25–4 μg/reaction), pro α1(I) and pro α2 are synthesized in a ratio of 2 to 1. However, at higher concentrations of RNA, the ratio decreased and, at 20 μG of RNA per reaction, this ratio dropped below 1 to 1 (Figure 4). For this reason we did not exceed 4 μg of RNA per reaction in our experiments.

Effects of Col 1(I) [Pro α1(I) Derived] Peptide. Addition of col 1(I) decreased the incorporation of proline in the translation system (Figure 5A) and reduced the formation of collagenous protein (Figure 5B). A similar degree of inhibition was observed when collagenous protein was estimated by using either immunoprecipitation or gel electrophoresis to resolve the pro α1(I) and pro α2 chains (Figure 6). The magnitude

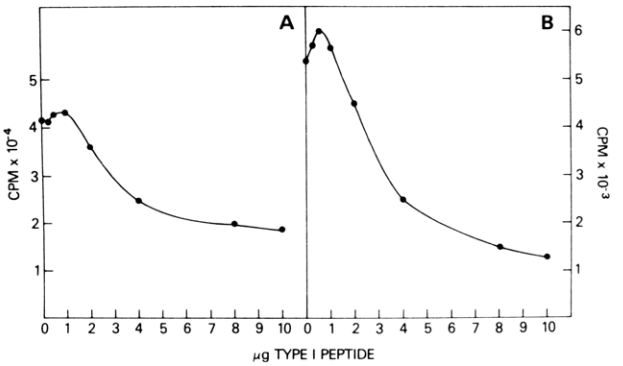

FIGURE 5: (A) Total incorporation of [^{3}H]proline into Cl_3AcOH-insoluble protein in the presence of increasing concentrations of col 1(I) peptide. (B) Immunoprecipitable incorporation from the reactions in (A). After removal of an aliquot for Cl_3AcOH precipitation and counting, the remainder was used for precipitation with type I procollagen specific immunoglobulins.

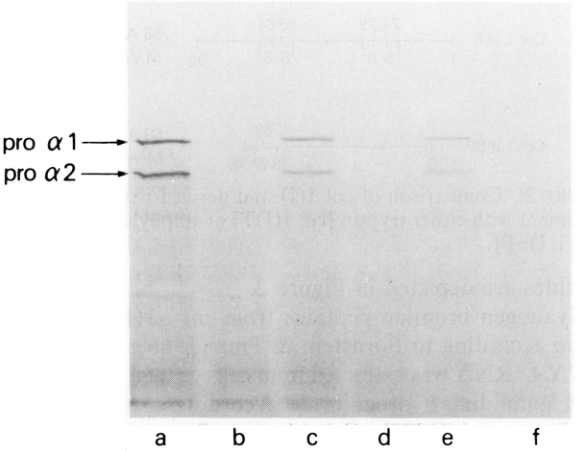

FIGURE 6: Slab gel electrophoresis of ^{14}C-labeled rat calvaria cell-free product. Each reaction was run with 4 μg of RNA per 125 μL of reaction. Following translation, duplicate reactions were treated with bacterial collagenase (tracks b, d, and f). Tracks c and d: reaction run with 5 μg of col 1(I) added. Tracks e and f: translation with 10 μg of col 1(I).

of the decrease observed in total protein synthesis, in terms of Cl_3AcOH-insoluble radioactivity, exceeded the change in immunoprecipitable protein radioactivity. This could represent an inability of the antibody to react with and precipitate incomplete chains, since the reduction in pro α1(I) and pro α2 chains appeared to be the major change observed in the electrophoretic pattern. In most experiments, the inhibition observed was not linear and tended to tail off at high levels of peptides. The formation of both pro α1(I) and pro α2 was inhibited by added peptides. The ratio of pro α1(I) to pro α2 remained 2 to 1 even when the production of collagenous protein was reduced 50% or more.

The effect of col 1(I) on translation with increasing amounts of calvaria RNA is shown in Figure 7. The results show that increasing amounts of RNA do not overcome the inhibition produced by this peptide. This would suggest that the inhibition produced in this system by col 1(I) is not dependent on the total RNA added to the system.

We next determined whether the inhibition by col 1(I) was specific for collagenous protein. By use of peptide concentrations of up to 4–6 μg (approximately 3–5 μM), little or no effect of col 1(I) was noted on the synthesis of noncollagenous proteins coded for calvaria RNA. Col 1(I) caused a slight increase in translation of RNA from reticulocytes in the range of 0.5–1 μg of peptide (less than 1 μM) but had little or no further effect on translation at higher levels (not shown).

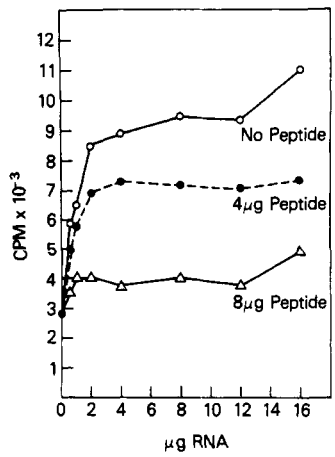

FIGURE 7: Cell-free translation with increasing amounts of rat calvaria RNA and at a constant concentration of col 1(I) peptide—either 0, 4, or 8 μg per reaction.

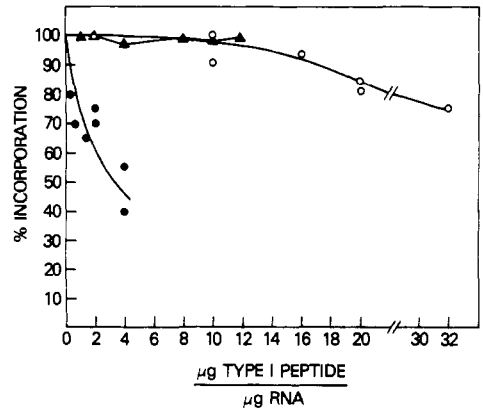

FIGURE 8: Comparison of col 1(I) peptide effect on rat calvaria RNA (●), globin RNA (○), and rat liver "vitellogenin" RNA (▲) translation. Each point represents an average of at least four determinations.

Approximately 85% of the protein coded for by this RNA is globin. The difference in the sensitivity of the globin RNA and calvaria RNA translation to NH_2-terminal peptide is shown in Figure 8. The calvaria RNA, with procollagen accounting for the majority of the [^{3}H]proline incorporation, is sensitive to lower concentrations of col 1(I). When globin RNA is used with either [^{3}H]proline or [^{35}S]methionine precursor (latter not shown), the peptide has little effect until very high concentrations are reached. Similar results were obtained when RNA from estrogen-primed liver was translated. Low levels of col 1(I) stimulated total incorporation slightly, with [^{35}S]methionine as the radioactive precursor, and some 15% of the protein coded for by this RNA is vitellogenin (Wetekam et al., 1975). After translation of the RNA, an approximately 200000 molecular weight protein was observed upon gel electrophoresis, as expected for vitellogenin. Formation of this peptide was not altered by col 1(I) at levels up to 16 μg/reaction. These observations indicate that the translation of mRNA for type I pro α chains is more sensitive to the inhibitory effects of col 1(I) than the mRNAs for the other proteins tested here.

Effects of Other Peptides. The disulfide-linked peptide prepared from the NH_2-terminal portion of type III procollagen is as inhibitory as an equal amount of col 1(I). Both reduce type I procollagen synthesis as determined by specific immunoprecipitation of cell-free product (Table I).

Three modified forms of col 1(I) were tested for activity, and all proved to have some degree of translational inhibition.

Table I

peptide	μg	μM	immunoprecipitable incorpn[a]	% decrease[b]
none			5308	
col 1(I)	2	1.6	3286	38.1
	4	3.2	1540	71.0
col 1(III)	2	0.4	2818	46.9
	4	0.8	2010	62.1
col 1(I)T	2	1.7	2726	48.6
	4	3.4	2716	48.8
col 1(I)SP	2	2.1	4484	15.5
	4	4.2	3560	32.9
col 1(I)RC	2	1.6	4141	22.0
	4	3.2	4013	24.4
α1(I) CB3	2	1.3	5434	0
	4	2.6	5298	0
α1(I) CB6	2	1.1	5270	0.7
	4	2.2	5195	2.1
α1(I) CB7	2	0.64	5438	0
	4	1.28	5502	0
α1(I) CB8	2	0.64	5240	1.3
	4	1.28	5382	0

[a] Data presented are from a representative experiment. Four separate determinations were made for each peptide. While differences in absolute incorporation existed between experiments, variability with respect to percent decrease from control values was less than 10% for NH_2-terminal peptides and less than 5% for cyanogen bromide derived peptides. Reactions were as described under Materials and Methods, using [^{3}H]proline. [b] Percent decrease is in relation to immunoprecipitable incorporation in the absence of added peptide.

The reduced and alkylated peptide [col 1(I)RC] was considerably less active as an inhibitor than the intact peptide (Table I). The trypsin-treated peptide [col 1(I)T] retained much of its activity while the staphylococcal protease treated peptide [col 1(I)SP] was less inhibitory than the intact col 1(I).

Several peptides, generated by cyanogen bromide digestion of the α1(I) chain, were tested for inhibitory activity and did not alter either the total incorporation of [^{3}H]proline into protein or the amount of newly synthesized protein precipitated by antibody to type I procollagen (Table I).

Discussion

While protein synthesis is ultimately controlled at the level of gene expression by selective transcription, further regulation at the translational level also probably exists. One example of translational control is the regulation of globin synthesis in reticulocytes by hemin. Addition of hemin to reticulocytes or cell lysates reduces the endogenous production of hemin while stimulating globin synthesis (Rabinovitz et al., 1969). With insufficient hemin, protein synthesis rapidly declines, polysomes disaggregate (Adamson et al., 1969), and there are reduced amounts of 40S Met-tRNA$_f$ complexes (Darnbrough et al., 1972). In hemin deficiency (Farrell et al., 1977), inhibition is at the level of polysome initiation and may be caused by the phosphorylation of initiation factor eIF2 by a specific protein kinase.

Another proposed method for specific translational control involves the interaction of low molecular weight RNAs with existing transcripts (Berns et al., 1975; Heywood et al., 1974). Either specific activation or inhibition by so-called "translational control RNA" has been described in several systems (Heywood & Kennedy, 1976; Zeichner & Breitkreutz, 1978); however, the mechanism through which they act is unclear.

The studies by Krieg et al. (1978) and Wiestner et al. (1979) have shown that peptides prepared from the N-terminal region of the pro α1(I) or pro α1(III) chain reduce collagen synthesis

when added to the media of cultured cells. These studies suggest that these peptides can influence the rate of collagen synthesis and even be a regulating factor. While the mechanism of action of these peptides at the cell level is not understood, we were encouraged to examine their effects on the translation of mRNA from calvaria in the reticulocyte cell-free system.

Our studies show that col 1(I) and col 1(III) inhibit the synthesis of both pro α1(I) and pro α2 chains in the cell-free system. The synthesis of other proteins coded for by the calvaria mRNA did not appear to be reduced. Additionally, these peptides did not reduce the synthesis of protein directed by reticuloyte RNA, primarily globin RNA, or by liver RNA in which vitellogen was a prominent product. The latter protein was tested because of its similarity to procollagen as a high molecular weight, processed, secretory protein. We wanted to eliminate the possibility of the peptide acting only on the translation of larger proteins. In general, these studies suggest that these peptides are rather specific in effecting the synthesis of pro α1(I) and pro α2 chains.

The fact that col 1(III) was as effective as col 1(I) in reducing type I procollagen synthesis is not surprising since both peptides have similar amino acid sequences and stable tertiary structure (Wiestner et al., 1979). This result does raise the question, however, of peptide specificity with regard to collagen type in vivo. It will be of interest to test the effects of these peptides on the translation of mRNA for other collagen types when they become available.

Col 1(I) represents only the first 98 residues of the peptide released from type I procollagen, since additional residues are removed by the collagenase treatment. It is possible that some differences in the activity or the specificity of the effect would be noted with the whole peptide.

Other peptides prepared from the α1(I) chain by CNBr digestion did not inhibit the translation of collagen. Further, the col 1(I) peptide after reduction and alkylation of disulfide bonds was much less inhibitory. It is known that the disulfide bonds in the intact peptide are necessary for antigenic recognition, suggesting that the peptide has a very specific conformation. The reduction in procollagen translation by col 1(I)T, with its chain disrupted but the disulfide bridges intact, further suggests a conformational specificity. The reduced inhibition by col 1(I)SP may be due to its significant reduction in size (30 residues) following enzymatic treatment.

The mechanism of inhibition is not clear. The translation of pro α1(I) and pro α2 is equally reduced. Further, additional RNA in the reaction mixture does not overcome the inhibition, suggesting that a direct interaction of peptide with mRNA is not involved. However, such an effect of added mRNA might be dependent on the rate of reinitiation of translation and this may not be optimal in our system. It is also possible that there are specific interactions between col 1(I) and col 1(III) with the nascent pro α chains and that this interaction slows translation.

Thus, these NH_2-terminal entension peptides have a marked effect on procollagen synthesis when added to either cells in culture or procollagen mRNA in a cell-free system. Whether these peptides have a similar function in vivo remains an important question; however, this regulatory system is a plausible mechanism for the cellular control of collagen synthesis.

References

Adamson, S. D., Herbert, E., & Kemp, S. F. (1969) *J. Mol. Biol. 42*, 247.

Becker, U., Timpl, R., Helle, O., & Prockop, D. J. (1976a) *Biochemistry 15*, 2853.

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

Berg, R. A., & Prockop, D. J. (1973) *Biochemistry 12*, 3395.

Berns, A., Salden, M., Bogdanovsky, D., Raymondjean, M., Shapira, G., & Bloemendal, H. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 714.

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

Bornstein, P., & Piez, K. (1966) *Biochemistry 5*, 3460.

Darnbrough, C., Hunt, T., & Jackson, R. J. (1972) *Biochem. Biophys. Res. Commun. 48*, 1556.

Farrell, P., Balkow, K., Hunt, T., Jackson, R. J., & Tachsel, H. (1977) *Cell 11*, 187.

Furthmayr, H., Timpl, R., Stark, M., Lapiere, C. M., & Kühn, K. (1972) *FEBS Lett. 28*, 247.

Heywood, S. M., & Kennedy, D. S. (1976) *Biochemistry 15*, 3314.

Heywood, S. M., Kennedy, D. S., & Bester, A. J. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 2428.

Jimenez, S. A., Dehm, P., Olsen, B. R., & Prockop, D. J. (1973) *J. Biol. Chem. 248*, 720.

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

Laemmli, U. K. (1970) *Nature (London) 227*, 680.

Lapiere, C. M., Lanears, A., & Kohn, L. D. (1971) *Proc. Natl. Acad. Sci. U.S.A. 68*, 3054.

Monson, J. M., & Goodman, H. M. (1978) *Biochemistry 17*, 5122.

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

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.

Rabinovitz, M., Freedman, M. L., Fisher, J. M., & Maxwell, C. R. (1969) *Cold Spring Harbor Symp. Quant. Biol. 34*, 567.

Rohde, H., Becker, U., Nowack, H., & Timpl, R. (1976) *Immunochemistry 13*, 967.

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

Smith, B. D., Byers, P. H., & Martin, G. R. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69*, 3260.

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

Weber, L. A., Hickey, E. D., Maroney, P. A., & Baglioni, C. (1977) *J. Biol. Chem. 252*, 4007.

Wetekam, W., Mullinix, K. P., Deeley, R. G., Kronenberg, H. M., Eldridge, J. D., Meyers, M., & Goldberger, R. F. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 3364.

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

Zeichner, M., & Brietkreutz, D. (1978) *Arch. Biochem. Biophys. 188*, 410.