Mutations That Alter the Primary Structure of Type I Procollagen Have Long-Range Effects on Its Cleavage by Procollagen *N*-Proteinase†

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ABSTRACT: Type I/II procollagen *N*-proteinase was partially purified from chick embryos and used to examine the rate of cleavage of a series of purified type I procollagens synthesized by fibroblasts from probands with heritable disorders of connective tissue. The rate of cleavage was normal with procollagen from a proband with osteogenesis imperfecta that was overmodified by posttranslational enzymes. Therefore, posttranslational overmodification of the protein does not in itself alter the rate of cleavage under the conditions of the assay employed. Cleavage of the procollagen, however, was altered in several procollagens with known mutations in primary structure. Two of the procollagens had in-frame deletions of 18 amino acids encoded by exons 11 and 33 of the proα2(I) gene. In both procollagens, both the proα1(I) and the proα2(I) chains were totally resistant to cleavage. With a procollagen in which glycine-907 of the α2(I) chain domain was substituted with aspartate, both proα chains were cleaved but at a markedly decreased rate. The results, therefore, establish that mutations that alter the primary structure of the proα chains of procollagen at sites far removed from the *N*-proteinase cleavage site can make the protein resistant to cleavage by the enzyme. The long-range effects of in-frame deletions or other changes in amino acid sequence are probably explained by their disruption of the hairpin structure that is formed by each of the three proα chains in the region containing the cleavage site and that is essential for cleavage of the procollagen molecule by *N*-proteinase.

The conversion of type I procollagen to collagen involves two different enzymes: a procollagen *N*-proteinase to remove the N-propeptides[1] (Kohn et al., 1974; Tuderman et al., 1978; Layman, 1981) and a procollagen C-proteinase to remove the C-propeptides (Goldberg et al., 1975; Kessler & Goldberg, 1978; Duksin et al., 1978; Leung et al., 1979; Njieha et al., 1982; Hojima et al., 1985; Kessler et al., 1986). Type I/II procollagen *N*-proteinase cleaves the N-propeptides from type I and type II procollagen, but not from the structurally similar type III procollagen (Tuderman et al., 1978; Tuderman & Prockop, 1982; Tanzawa et al., 1985). A separate *N*-proteinase has been shown to cleave the N-propeptides from type III procollagen (Nusgens et al., 1980; Halila & Peltonen, 1984, 1986).

The reaction with type I/II *N*-proteinase requires that the procollagen substrate be in the correct three-dimensional conformation (Tuderman et al., 1978). The protein totally resists cleavage if it is denatured or separated into proα chains (Tuderman et al., 1978; Tuderman & Prockop, 1982; Tanzawa et al., 1985). Also, short synthetic peptides with amino acid sequences identical with those in the cleavage site of the proα1(I) chain are not cleaved (Morikawa et al., 1980).

Three probands with heritable disorders of connective tissue were shown to have mutations that alter the *N*-proteinase cleavage site in type I procollagen so as to make the protein resistant to cleavage by the enzyme. Uncleaved pNα2(I) chains were observed in skin extracts of a proband with type VII EDS (Steinmann et al., 1980), who was subsequently shown to have a mutation that produced an in-frame deletion of the 18 amino acids that are encoded by the telopeptide junction exon 6[2] of the proα2(I) gene and that contain the cleavage site of the proα2(I) chain (Wirtz et al., 1987). Similarly, uncleaved pNα1(I) chains were present in skin extracts of a second proband with EDS VII that had an in-frame deletion of the 24 amino acids that are encoded by the telopeptide junction exon 6 of the proα1(I) gene and that contain the cleavage site of the proα1(I) chain (Cole et al., 1986). In a third proband with type VII EDS, there was evidence for a failure to cleave proα2(I) chains because of a similar deletion of amino acids around the cleavage site of the proα2(I) chain (Eyre et al., 1985).

In addition, preliminary observations with procollagens from two probands with OI suggested that in-frame deletions of amino acids at sites far removed from the *N*-proteinase cleavage site can make the protein resistant to cleavage (Williams & Prockop, 1983; Sippola et al., 1985). Here we

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[1] Abbreviations: N- and C-propeptides, amino- and carboxy-terminal propeptides, respectively, of procollagen; OI, osteogenesis imperfecta; EDS, Ehlers–Danlos syndrome; DMEM, Dulbecco's modified Eagle's medium; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; DEAE, diethylaminoethyl; EDTA, ehtylenediaminetetraacetic acid; SDS, sodium dodecyl sulfate; pCcollagen, partially processed procollagen containing only the C-propeptides; pNcollagen, partially processed procollagen containing only the N-propeptides.

[2] Exons of the proα1(I) and proα2(I) genes are numbered by the convention that assigns the same number to comparable domains of all fibrillar collagens. Therefore, the telopeptide junction exon containing the *N*-proteinase cleavage site is numbered exon 6, and the telopeptide junction exon containing the C-proteinase cleavage site is numbered exon 49. As a result, exons 33 and 34 that are separate exons in the genes for other fibrillar genes are referred to as a fused exon 33/34 in the proα1(I) gene.

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have examined cleavage by type I/II *N*-proteinase of a series of procollagens in which mutations that change the primary structure of the protein were recently defined.

Materials and Methods

Preparation of N-Proteinase. For most of the experiments, type I/II procollagen *N*-proteinase was purified from 13-day whole chick embryos using a four-step procedure that was based on previously published procedures (Tuderman & Prockop, 1982; Berger et al., 1985; Tanzawa et al., 1985) and that included extraction of a homogenate of whole chick embryos, ammonium sulfate precipitation of the crude extract, and chromatography on concanavalin A–Sepharose and heparin–Sepharose (Dombrowski & Prockop, 1988). The procedure provided enzyme purified over 1500-fold and with an average specific activity of about 300 units/mL[2]. Several experiments were carried out with procollagen *N*-proteinase purified with a similar procedure from chick embryo tendons (Tuderman & Prockop, 1982; Y. Hojima and D. J. Prockop, unpublished results).

Preparation of Procollagen Substrates. Radioactively labeled type I procollagen was purified from the medium of cultured human skin fibroblasts from three patients with OI (CRL-1262, American Tissue Type Collection; RMS-2, RMS-42, and RMS-69), one patient with EDS (RMS-44), and three patients exhibiting no clinical or pathological symptoms of a connective tissue disorder (GM 3349 and GM 3348 from the Human Genetic Mutant Cell Repository, and JIMM-86). Cells were grown under standard conditions (Williams & Prockop, 1982; de Wet et al., 1983; Deak et al., 1983; Sippola et al., 1984). At confluency, the cells were incubated twice for 24-h periods with 1 μCi/mL ^{14}C-labeled amino acids in DMEM containing 25 μg/mL ascorbic acid. The ^{14}C-labeled procollagen was precipitated from the medium by ammonium sulfate, and the precipitated protein was suspended in 10 mL of a 0.1 M Tris-HCl buffer, pH 7.5, containing 2 M deionized urea and 0.2 M NaCl. The procollagen was purified on two successive columns of DEAE-cellulose (Peltonen et al., 1980; Fiedler-Nagy et al., 1981). The pooled fractions containing type I procollagen were dialyzed against a storage buffer that contained 0.4 M NaCl in 0.1 M Tris-HCl, pH 7.4, and was concentrated by using a YM-100 DIAFLO ultrafiltration membrane (Amicon Corp., Danvers, MA). Procollagen concentration was determined by colorimetric hydroxyproline assay (Kivirikko et al., 1967) and assuming 10.11% hydroxyproline by weight for type I procollagen (Fiedler-Nagy et al., 1981). The specific activities of the ^{14}C-labeled procollagen substrates were as follows: normal (JIMM-86, GM 3346, GM 3349), 2600–2800 cpm/μg; RMS-2, 1600 cpm/μg; RMS-42, 1050 cpm/μg; RMS-44, 1000 cpm/μg; CRL-1274, 2600 cpm/μg; RMS-69, 1600 cpm/μg.

All fibroblasts that synthesized proα chains with a decreased electrophoretic mobility were incubated with 0.3 mM α,α'-dipyridyl to establish that the decreased mobility was caused by posttranslational overmodification (Williams & Prockop, 1982).

N-Proteinase Assay. The procollagens were assayed as substrates for procollagen *N*-proteinase with the gel electrophoresis assay method (Berger et al., 1985; Dombrowski et al., 1986). The standard assay was carried out in a volume of 100 μL that contained 1 μg of ^{14}C-labeled type I procollagen, 5 mM $CaCl_2$, and 1 unit of *N*-proteinase in a 50 mM Tris-HCl buffer (pH 7.4 at 30 °C) with a final NaCl concentration of 150–200 mM. As noted, a few assays were carried out with 0.5 unit of enzyme. The reaction mixture was incubated at 30 °C for the times indicated, and the reaction was stopped by the addition of 25 μL of a 50 mM Tris-HCl buffer (pH 7.4 at 30 °C) containing 150 mM NaCl and 500 mM EDTA.

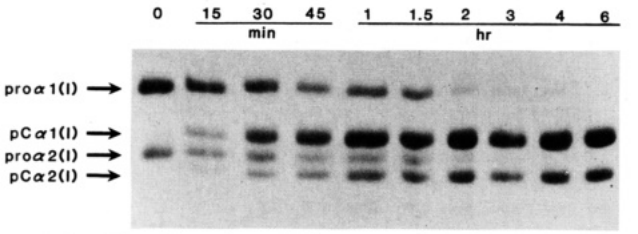

FIGURE 1: *N*-Proteinase cleavage of normal and genetically shortened human procollagen. Gel electrophoresis assay of the cleavage of human type I/II procollagen (GM 3349) by *N*-proteinase. the cleavage products were separated by using a 6% polyacrylamide separating gel and a 4% polyacrylamide stacking gel.

Table I: Summary of Procollagen Defects

cell line	clinical phenotype	molecular defect	overmodification	% cleaved by *N*-proteinase[a]
controls[a]	normal	none	none	100 ± 2
RMS-44[b]	EDS/OI	proα2^{-Ex11}	none	0
RMS-42[c]	OI-II	proα2^{-Ex33}	moderate	0
RMS-69[d]	OI-II	proα2$^{Gly907\rightarrow Asp}$	marked	63 ± 11[f]
RMS-2[e]	OI-II	unknown	marked	90 ± 4
			moderate	100 ± 2

[a] Values are mean ± standard error of the mean for percent of collagen proα chains cleaved in 4 h under standard assay conditions with 1 unit of *N*-proteinase. A total of 3–9 assays were performed with procollagen from the same cell line. The control cell lines were GM 3348, GM 3349, and JIMM-86. Values for the mutated procollagens are for the shortened or overmodified proα1(I) chains assayed by densitometry of the appropriate bands (see Figure 3). [b] Shortened proα2-(I) chain because of a 19 bp deletion that causes splicing out of 18 amino acids (amino acid residues 73–90) encoded by exon 11 (Sippola et al., 1984; Kuivaniemi et al., 1988). [c] Type I procollagen in which half the proα2(I) chains contain a deletion of the 18 amino acids (amino acid residues 568–585) encoded by exon 33 (Pribula, 1985; Baldwin et al., 1988a). [d] Type I procollagen in which glycine-907 of the α2(I) chain is substituted with aspartate (Baldwin et al., 1988b). [e] Type I procollagen in which several species of proα chains are overmodified (see Figure 3). [f] Assayed with 0.5 units of *N*-proteinase extracted from chick embryo tendons, and therefore values are adjusted by a factor of 1.39 (see text).

Polyacrylamide Gel Electrophoresis. For examination of the reaction products by gel electrophoresis, 0.1 volume of 20% SDS and 0.2 volume of a 5×-concentrated sample buffer (Dombrowski et al., 1986) were added, and the sample was heated at 100 °C for 3 min. To reduce the protein prior to electrophoresis, 2-mercaptoethanol was added to a final concentration of 2%, and the sample was again heated at 100 °C for 3 min. Electrophoresis was on polyacrylamide slabs of 1.5-mm thickness with a stacking gel of 4% polyacrylamide and a separating gel of either 5% or 6% polyacrylamide. Fluorograms of the gels were prepared using Autofluor (National Diagnostics, Sommerville, NJ). Multiple exposures of autoradiograms were scanned by a Ultrascan XL laser densitometer spectrophotometer (LKB) to ensure linear response of the film.

Results

Normal Cleavage Of Posttranslationally Overmodified Type I Procollagen. Under the standard assay conditions developed here, the cleavage of normal human procollagen by 1 unit of *N*-proteinase was 45% ± 3% (SEM) complete in 30 min, and it was 100% ± 2% complete in 4 h (Figure 1 and

Table I). With 0.5 unit of enzyme,[3] 72% ± 3% of the substrate was cleaved in 4 h (not shown). The first question explored here was whether the posttranslational overmodification of procollagen frequently seen in OI and EDS (Prockop & Kivirikko, 1984; Byers & Bonadio, 1985; Prockop et al.; 1988) affects cleavage of the protein by procollagen N-proteinase. Type I procollagen was purified from the medium of fibroblasts from a lethal variant of OI (CRL-1262) that was previously shown to have a sporadic deletion of exons 26–28 from the middle of one allele to the proα1(I) chain (Chu et al., 1983, 1985; Barsh et al., 1985). The cell layer from the proband's fibroblasts contained procollagen with shortened proα1(I) chains (Barsh & Byers, 1981; Williams & Prockop, 1983). The medium of the fibroblast culture essentially contained none of the shortened chains. Instead, it contained highly overmodified type I procollagen. Barsh and Byers (1981) suggested that the overmodified type procollagen in the medium contained a second altered proα chain, but Williams and Prockop (1983) suggested it might be an overmodified type I procollagen with a normal primary structure. The procollagen from the cell layer containing the shortened proα1(I) chain resisted cleavage (Williams & Prockop, 1983). The overmodified type I procollagen purified from the medium was cleaved at a normal rate (not shown). Therfore, the results did not provide any evidence for a second mutation. Also, they demonstrated that overmodification of type I procollagen does not in itself make the protein resistant to N-proteinase.

Two Deletions in the Proα2(I) Chain That Make Both Proα Chains Totally Resistant to Cleavage. The rate of cleavage was examined with 2 procollagens shown to have in-frame deletions of 18 amino acids each at different sites in the proα2(I) chain.

The first procollagen (RMS-44 in Table I) had an in-frame deletion of the 18 amino acids encoded by exon 11 (amino acids 73–90)[4] of the proα2(I) gene (Sippola et al., 1984; de Wet et al., 1986; Kuivaniemi et al., 1988). The proband had an atypical form of OI with clinical features of both EDS and OI. She was heterozygous for the mutation, and the medium from her fibroblasts contained a mixture of normal type I procollagen and type I procollagen containing the shortened proα2(I) chains (Sippola et al., 1984). However, the procollagen containing the shortened proα2(I) chains was selectively lost during the purification procedures employed here. Hence, the final preparations contained about 75% normal type I procollagen and about 25% of the mutated procollagen. The normal procollagen was cleaved at a normal rate (Figure 2). In contrast, the procollagen containing the shortened proα2(I) chains resisted cleavage (Table I). Again, both the proα1(I) and proα2(I) chains resisted cleavage (not shown) even though the structural alteration was present only in the proα2(I) chains.

The second procollagen (RMS-42 in Table I) had an in-frame deletion of the 18 amino acids encoded by exon 33 (amino acids 568–585) of the proα2(I) gene (Pribula, 1985; Baldwin et al., 1988a). Again, the proband had a lethal variant of OI and was heterozygous for the mutation. The procollagen containing the abnormal proα2(I) chain was overmodified, and most was retained in the cell layer of fibroblast cultures (Pribula, 1985). Hence, for the experiments here, the cell layer proteins were only partially purified by precipitation of cell homogenates with ammonium sulfate, and they were used directly as substrate for N-proteinase. Because a 24-h labeling period was used and because the abnormal procollagen was secreted more slowly than normal type I procollagen, the overmodified procollagen containing the shortened proα2(I) chains accounted for about 75% of the total type I procollagen in the sample. As indicated in Figure 2, about 25% of the total procollagen was digested at a normal rate. The proα chains that were cleaved all had a normal electrophoretic mobility (not shown). The overmodified procollagen resisted digestion. Again, both the proα1(I) and proα2(I) chains were totally resistant to digestion during the 10-h incubation period (Table I).

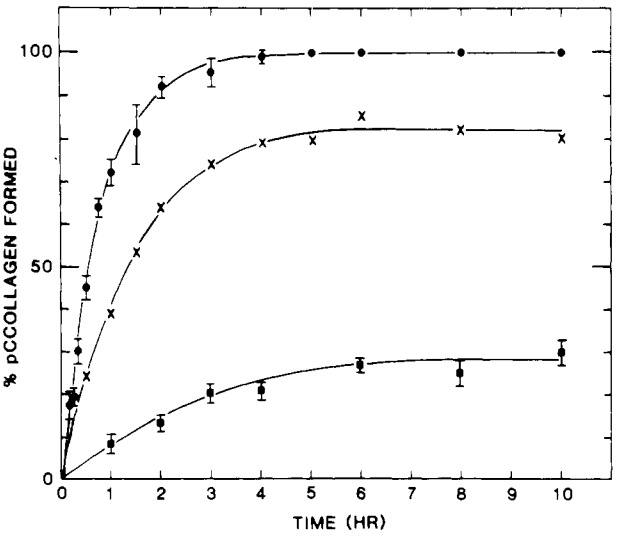

FIGURE 2: Time course for cleavage of normal procollagen and three procollagens containing in-frame deletions in the proα2(I) chain. Type I procollagens were purified from the media of fibroblast cultures for all samples except RMS-42. Because of poor secretion of the altered procollagen, a cell layer extract was used for RMS-42. the percent conversion of type I procollagen to pCcollagen was calculated as 100 × the ratio of the areas of the sum of the pCα1(I) and pCα2(I) peaks to the sum of the areas of the proα1(I), proα2(I), pCα1(I), and pCα2(I) peaks. (●) Observed percent conversion ± standard error of the mean for three normal procollagens (GM 3348, GM 3349, and JIMM-86; $n = 9$); (×) procollagen containing deletion of 18 amino acids encoded by exon 11 of the proα2(I) gene (RMS-44, $n = 2$); (■) procollagen containing deletion of the 18 amino acids encoded by exon 33 of the proα2(I) gene (RMS-42, $n = 4$). The sample of procollagen from RMS-44 consisted of about 75% normal procollagen and about 25% mutated procollagen containing a shorter proα2(I) chain. The proportions were reversed in the sample of procollagen from RMS-42.

A Mutation That Converts Glycine-907 of the α2(I) Chain to Aspartate Decreases the Rate of Cleavage. Procollagen was also examined from a lethal variant of OI that was recently shown to have a single base mutation that converted glycine-907 of the α2(I) chain to aspartate (Baldwin et al., 1988b). Medium from the proband's fibroblasts contained a mixture of normal procollagen and overmodified procollagen containing the aspartate for glycine substitution. The normal procollagen was cleaved at the same rate as procollagen from control fibroblasts (not shown), but the overmodified procollagen containing the mutation was cleaved at a slower rate (RMS-69 in Table I). The rates of cleavage of both the overmodified proα1(I) chains and the overmodified proα2(I)

[3] One unit of N-proteinase activity is defined at 0.5 μg of chick type I procollagen cleaved in 30 min at 35 °C under standard assay conditions (Berger et. at., 1985) and is equivalent to 3 units in an assay using sheep pNcollagen as substrate (Dombrowski et. al., 1986).

[4] Amino acid positions are numbered by the standard convention in which the first glycine of the triple-helical domain of an α chain is number 1. The numbers for α1(I) chains can be converted to positions in the human proα1(I) chain by adding 156, and numbers for the α2(I) chain can be converted to the human proα2(I) chain by adding 68. The 18 amino acids deleted from the proα2(I) chain in RMS-44 are amino acids 73–90. The 18 amino acids deleted from the proα2(I) chain in RMS-42 are amino acids 568–585.

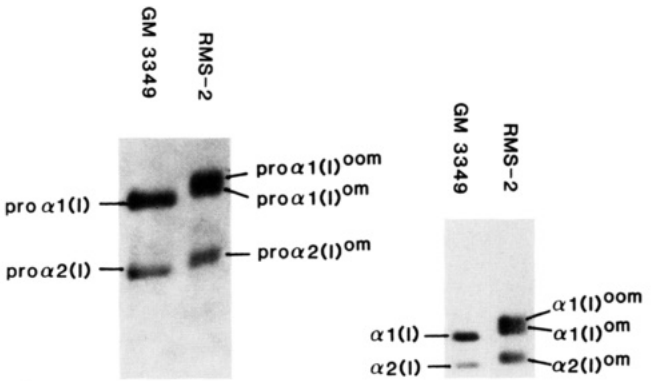

FIGURE 3: Overmodified type I procollagen from the OI cell line (RMS-2). (Left panel) Procollagen from the cell layer of control (GM 3349) and OI (RMS-2) fibroblasts. Cultured cells wee incubated for 4 h with a ^{14}C-labeled amino acid mixture as described under Materials and Methods, and proteins were separated on a 5% polyacrylamide gel. (Right panel) Same procollagens after digestion with pepsin. Abbreviations: om, posttranslational overmodification; oom, over-overmodification.

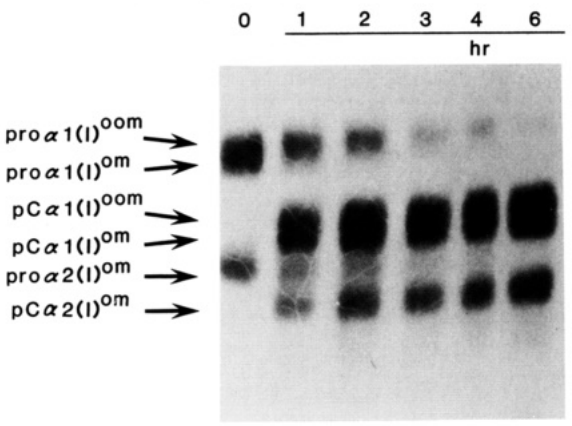

FIGURE 4: Differential cleavage of proα1(I) chains by *N*-proteinase. Gel electrophoresis assay of the cleavage of purified ^{14}C-labeled type I procollagen with an unidentified alteration in structure (RMS-2). Cleavage products were separated on a gel of 5% polyacrylamide. C denotes control incubated for 10 h at 30 °C with no enzyme; om denotes posttranslational overmodification; oom denotes over-overmodification.

chains were essentially the same (not shown).

An Undefined Mutation That Causes Synthesis of an Overmodified Procollagen That Is Cleaved Normally and an Overmodified Procollagen That Is Cleaved Slowly. We next examined procollagen from a proband with a lethal variant of OI with a still undefined mutation. Procollagen from the medium of the proband's fibroblasts contained two distinct bands of overmodified proα1(I) chains and one band of overmodified proα2(I) chains (Figure 3). Comparison with several controls indicated that none of the type I proα chains in the medium comigrated with proα chains of normal type I procollagen. All the proα chains migrated normally when the fibroblasts were incubated with 0.3 mM α,α′-dipyridyl (not shown). The lower band of proα1(I) chains and the proα2(I) chains were cleaved at a normal rate (Figures 4 and 5 and Table I). The upper band of proα1(I) chains was cleaved more slowly (Figure 4 and Table I). Therefore, the results suggested that the procollagen had an alteration in structure in the proα1(I) chain. The apparently normal rate of cleavage of proα2(I) chains may be explained by earlier observations indicating that the proα chains are cleaved sequentially and that the proα2(I) chain is cleaved before the second proα1(I) chain (Berger et al., 1985).

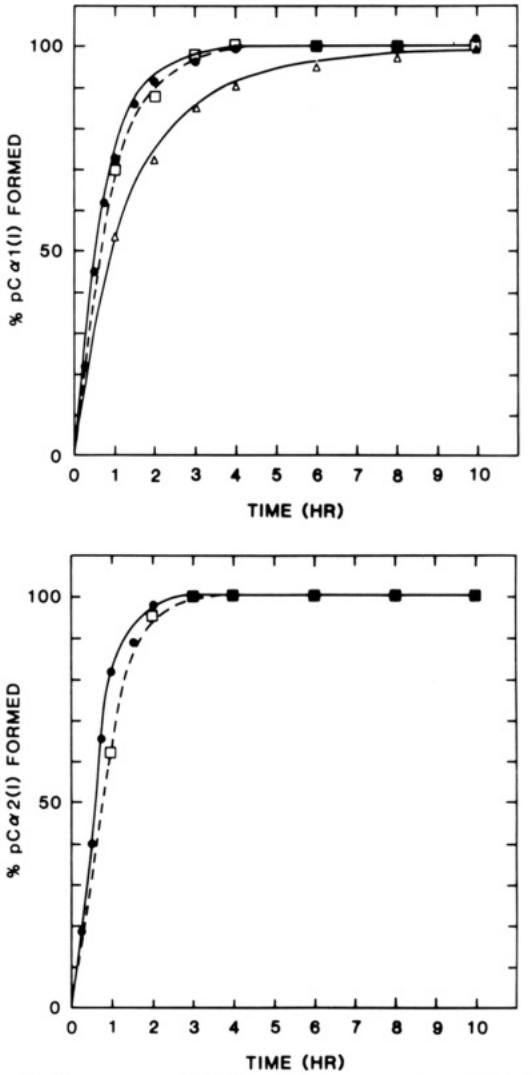

FIGURE 5: Time course of differential cleavage of proα1(I) chains by *N*-proteinase. The percent conversion of proα1(I) chains to pCα1(I) chains was calculated as 100 × the ratio of the area of the pCα1(I) peak to the sum of the areas of the proα1(I) and pCα1(I) peaks and expressed as the average of three experiments. The percent conversion of proα2(I) chains was calculated in a similar manner. (●) Cleavage of normal procollagen (GM 3349); (□) overmodified procollagen with unidentified alteration in structure (RMS-2); (△) over-overmodified procollagen with unidentified alteration in structure (RMS-2).

Discussion

Previous reports establish that type I/II procollagen *N*-proteinase is unusual among proteinases in that it requires a procollagen substrate with a native conformation (Tuderman et al., 1970; Tuderman & Prockop, 1982; Berger et al., 1985; Tanzawa et al., 1985). Type III *N*-proteinase has a similar requirement (Hallila & Peltonen, 1984, 1986). The results here establish that small changes in the primary structure of type I procollagen can either make the protein totally resistant to cleavage or substantially alter the rate at which it is cleaved by the type I/II *N*-proteinase. The changes in primary structure can occur at sites over 900 amino acid residues (about 300 nm) away from the cleavage site. Also, the changes can be as small as a single amino acid substitution. The long-range effects of changes in primary structure are probably explained by their disruption of the hairpin structure that is formed by each of the three proα chains in the region containing the cleavage site of the type I procollagen molecule (Helseth et al., 1981; Helseth & Veis, 1981; Dombrowski & Prockop, 1988).

The 3 procollagens containing in-frame deletions of 18 amino acids each at different sites in the proα2(I) chain made all three proα chains in the molecule totally resistant to cleavage. The results, therefore, are consistent with the suggestion that the conformation of the cleavage site is stabilized by lateral interactions of the three hairpins formed by the proα chains (Dombrowski & Prockop, 1988). In-frame deletions of -Gly-X-Y-sequences anywhere within the triple-helical domain of one of the proα chains probably cause slippage and misregistration of the three chains at the cleavage site. The resulting unfolding of one of the hairpin structures probably disrupts the lateral interactions and thereby induces cooperative unfolding of the complete structure.

The decreased rate of cleavage of the procollagen containing the aspartate substitution for glycine-907 is probably explained by the mutation causing a change in phase of the three proα chains in the triple-helical domain of the protein that alters the conformation of the *N*-proteinase cleavage site. Recently, we prepared molecular models incorporating phase shifts for a mutated procollagen in which cysteine was substituted for the glycine in position 748 of the α1(I) chain (Vogel et al., 1988). The models predicted a flexible kink in the triple helix, and we subsequently demonstrated the presence of a kink at the site of the mutation by rotary-shadowing electron microscopy of the protein (Vogel et al., 1988). Unfortunately, the aspartate for glycine substitution examined here is probably too close to the C-terminus of the molecule and too close to the junction with the C-propeptide to be discernible by electron microscopy as a site of increased flexibility (Hofmann et al., 1974).

The observations here also established that posttranslational overmodification of the protein does not, in itself, alter the rate at which it is cleaved by *N*-proteinase. The species of overmodified procollagens that were examined may well have mutations in structure that affect the rate at which they fold during biosynthesis but do not significantly alter the conformation of the cleavage site.

The sensitivity of the *N*-proteinase to alterations in the primary structure of procollagen obviously provides a powerful technique of searching for mutations that change the protein. The observations with procollagen with a still unidentified mutation (RMS-2 in Table I and Figure 4) indicated that only the proα1(I) chain was cleaved more slowly than the control. Therfore, in some instances, assay with the enzyme may make it possible to determine which of the two chains contains the mutation and reduce the amount of effort necessary to define the mutation.

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Registry No. Type I/II procollagen *N*-proteinase, 63651-94-5.

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Chemical Modification of the Functional Arginine Residues of Carbon Monoxide Dehydrogenase from *Clostridium thermoaceticum*†

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ABSTRACT: Carbon monoxide dehydrogenase (CODH) is the key enzyme of autotrophic growth with CO or CO_2 and H_2 by the acetyl-CoA pathway. The enzyme from *Clostridium thermoaceticum* catalyzes the formation of acetyl-CoA from the methyl, carbonyl, and CoA groups and has separate binding sites for these moieties. In this study, we have determined the role of arginine residues in binding of CoA by CODH. Phenylglyoxal, an arginine-specific reagent, inactivated CODH, and CoA afforded about 80–85% protection against this inactivation. The other ligands, such as the carbonyl and the methyl groups, gave no protection. By circular dichroism, it was shown that the loss of activity is not due to extensive structural changes in CODH. Earlier, we showed that tryptophan residues are located at the CoA binding site of CODH [Shanmugasundaram, T., Kumar, G. K., & Wood, H. G. (1988) *Biochemistry 27*, 6499–6503]. A comparison of the fluorescence spectra of the native and phenylglyoxal-modified enzymes indicates that the reactive arginine residues appear to be located close to fluorescing tryptophans. Fluorescence spectral studies with CoA analogues or its components showed that CoA interacts with the tryptophan(s) of CODH through its adenine moiety. In addition, evidence is presented that the arginines interact with the pyrophosphate moiety of CoA.

Wood and his collaborators discovered a pathway of acetyl coenzyme A (acetyl-CoA)[1] synthesis in *Clostridium thermoaceticum* which differs from all previously described pathways for autotrophic growth such as the Calvin cycle or the reductive citric acid cycle. Most of the enzymes involved in various steps of the pathway have been purified [see Wood et al. (1986a–c) and Ljungdahl (1986) for reviews]. Carbon monoxide de-

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[1] Abbreviations: acetyl-CoA, acetyl coenzyme A; CODH, carbon monoxide dehydrogenase; DTT, dithiothreitol; CoA, coenzyme A; CD, circular dichroism; DNPS-Cl, 2,4-dinitrophenylsulfenyl chloride.

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