A Post-translational Modification, Unrelated to Hydroxylation, in the Collagenous Domain of Nonhelical Pro-α2(I) Procollagen Chains Secreted by Chemically Transformed Hamster Fibroblasts

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Transformed Syrian hamster embryo (NQT-SHE) fibroblasts do not synthesize the pro-α1 subunit of type I procollagen, but secrete two modified forms of the pro-α2(I) subunit that migrate more slowly than the normal chain during gel electrophoresis (Peterkofsky, B., and Prather, W. (1986) J. Biol. Chem. 261, 16818–16826). By electrophoretic analysis of cyanogen bromide and V8 protease-derived peptides from the collagenous domains of intra- and extracellular pro-α2(I) chains, we find that the modification occurs almost exclusively in secreted molecules, is located in the region spanned by the cyanogen bromide peptide CB3,5, and persists when hydroxylation is inhibited. Thus, modification is due to a post-translational reaction other than hydroxylation. The modified chains appear to be secreted in the denatured state since: 1) helical structures formed at 4°C under acidic conditions were unstable under neutral conditions at 37°C; 2) conditions that destabilize the type I procollagen helix and thus inhibit its secretion, i.e. inhibition of proline hydroxylation or incorporation of the proline analog cis-hydroxyproline, did not affect secretion of the modified chains. The time courses for secretion of nonhelical modified chains from NQT-SHE and of hydroxylated helical procollagen I from control cells, as a proportion of total collagen synthesized, were similar. Although cis-hydroxyproline did not inhibit the secretion of the modified chains, it induced their rapid intracellular degradation.

Type I collagen is widely distributed in tissues of animals and is found as triple helical molecules in fibers of the extracellular matrix (1). The helix consists of two α1(I) and one α2(I) subunits that have repeating Gly-X-Y sequences with amino acids in the Y position (1,2). A precursor procollagen molecule that contains the helical collagenous domain, as well as globular domains at the carboxy- and amino-terminal regions, is synthesized in the endoplasmic reticulum, where formation of the hydroxylated amino acids also occurs (2–4). Hydroxyproline stabilizes the helical structure which is preferentially secreted (4). Both hydroxylations require ascorbate, iron, oxygen, and α-ketoglutarate (4).

We previously described a 4-nitroquinoline-1-oxide-transformed Syrian hamster embryo cell line (NQT-SHE) that did not synthesize the pro-α1(I) subunit of type I collagen but synthesized and secreted two polypeptides related to the pro-α2(I) subunit (5). The chains could be separated by ammonium sulfate fractionation at 33 and 50% saturation (5). Failure to synthesize the pro-α1(I) subunit appears to be related to the absence of mRNA for this polypeptide (6). The pro-α2(I) chains secreted by the transformed cells migrated more slowly than the normal pro-α2(I) polypeptide during sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE), and the α-chains derived from them by pepsin digestion retained the modified behavior.

In the present studies, we examined the basis for this modified behavior. Since 4-nitroquinoline-1-oxide can act as a mutagen as well as a carcinogen (7), it seemed possible that the polypeptides might be altered because of a mutation in the pro-α2(I) gene. A point mutation in the β-actin gene of a nitroquinoline-oxide-transformed human fibroblast line produced a single amino acid change that caused slower migration of the polypeptide during SDS-PAGE (8). The modified behavior of the pro-α2(I) chains produced by NQT-SHE cells also could be caused by hyper-hydroxylation. Normally, hydroxylation stops once sufficient hydroxyproline is produced to allow helix formation (4), but some of the prolyl and lysyl residues in the Y position may remain unhydroxylated (9). If type I procollagen chains remain denatured in the endoplasmic reticulum, then hydroxylation can continue. This effect is observed when a mutation that destabilizes the helical structure occurs in either of the subunits of type I procollagen produced by fibroblasts from patients with osteogenesis imperfecta (10–12). As a consequence, both subunits migrate more slowly than normal chains during SDS-PAGE (10–12). Since α2(I) chains by themselves form an unstable helical structure compared to either the type I heterotrimer or the α1(I) homotrimer (13, 14), hyper-hydroxylation of the pro-α2(I) chains produced by NQT-SHE fibroblasts might occur. Both of these possibilities were examined.

Because of the instability of the α2(I) trimer (13, 14), it has been assumed that in the absence of the pro-α1(I) chain, the pro-α2(I) chain would not be secreted. The pro-α2(I) chain was not detected in cultured mutant mouse cells that do not express the pro-α1(I) gene (14) or in human fibroblast cultures where a mutant pro-α2(I) chain is produced that cannot be incorporated into the type I procollagen helix (16), although in both cases mRNA for pro-α2(I) was expressed. Therefore, we determined the stability of the helix formed by the modified pro-α2(I) chains and whether secretion of these chains was altered.

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1 The abbreviations used are: NQT-SHE, 4-nitroquinoline-1-oxide transformed Syrian hamster embryo; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; BHK, baby hamster kidney; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.
would be affected by factors that inhibit type I procollagen secretion through destabilization of the triple helical structure.

EXPERIMENTAL PROCEDURES

Materials—The sources of radioactive isotopes, most chemicals, culture media, and culturing of NQT-SHE, primary SHE fibroblasts, and BHK-21 cells, an established line of Syrian hamster fibroblasts, have been described previously (5, 6). Both SHE and BHK fibroblasts produce normal type I procollagen, but SHE fibroblasts cannot be used beyond passage 5, while BHK fibroblasts can be continuously cultured. cis-4-Hydroxyproline was obtained from Behring Diagnostics and monensin was from Sigma.

Measurement of Collagen Synthesis and Preparation of Radioactive Procollagens—Growth medium was removed from late logarithmic phase cultures in 60- or 100-mm dishes. The cells were incubated for 24 h in fresh medium of NQT-SHE cells, which was continued for varying time intervals, as indicated in the legends to figures or tables. Incorporation of radioisotopes into collagen was measured by digestion of trichloroacetic acid-precipitated proteins with purified bacterial collagenase, as described previously (17). The extent of proline hydroxylation in collagen was measured in cells double labeled with [4-3H]- and [14C]proline by determining the H:14C ratio in the collagenase digest, as described previously (17).

Procollagens labeled with [3H]proline were isolated from cell and medium fractions by ammonium sulfate fractionation in the presence of protease inhibitors, as described previously (5). In the case of SHE and BHK fibroblasts, cells that produce mainly type I procollagen, precipitation was carried out at 35% saturation. For NQT-SHE cells, fractionation was carried out at 33 and 50% saturation to give P33 and P50 precipitated fractions (5). The modified pro-a2(I) chains in these fractions are referred to as N33 and N50 chains. Pepsin digestion of procollagens to form a-chains was carried out at 4 °C for 2 h at a protein:pepsin ratio of 14:1 (5), and the modified pro-a2(I) chains derived from the N-collagens are referred to as N33a and N50a. The P33 fraction from NQT-SHE cells also contained small amounts of types IV and V collagens.

SDS-PAGE and Peptide Mapping—SDS-PAGE under reducing conditions, isolation of a-chains from gels, and subsequent analysis of the excised polypeptides by peptide mapping were carried out as described previously (5) with a few modifications. Gels were longer (14 × 16 cm) than those used previously (12 × 14 cm) and 5% gels were used to separate a-chains for peptide mapping. Cyanogen bromide peptides derived from excised a-chains were electrophoresed on 8% instead of 10% gels and V8 protease-derived peptides were analyzed on 10% instead of 14% gels. These modifications resulted in better resolution of the modified a-chains and peptides derived from them. Gels were dried and fluorograms were prepared from them as described previously (5).

Melting Temperature Measurements—The stability of collagen helical structures was measured by a modification of a trypsin-resistance assay (11). Collagens obtained by pepsin digestion of [14C]proline-labeled procollagens were dissolved in 0.2 M NaCl, 0.05 M Hepes buffer, pH 7.2. After removal of the initial sample, the solution was equilibrated to the starting temperature (5 or 30 °C) for 20 min, a 10-μl portion was removed and equilibrated to 10 or 20 °C for 1 min, and then 10 μl of trypsin, 100 or 200 μg/ml, was added and the samples were incubated for an additional 2 min. Soybean trypsin inhibitor (10 μl of 100 or 200 μg/ml) was added immediately, followed by 30 μl of twice concentrated denaturing solution (20% sucrose, 4% SDS, 0.004% bromphenol blue, 0.5 M urea, and 20 mM dithiothreitol) and the samples were heated at 100 °C for 5 min. The remainder of the collagen solution was immediately equilibrated for 20 min at a temperature either 2 or 5 °C higher and the sampling process was repeated over temperature ranges indicated in Fig. 1. The a-chains were analyzed by SDS-PAGE on 5% gels on as described above. For the initial sample, soybean trypsin inhibitor was added prior to trypsin, then twice concentrated denaturing solution was added and the samples were heated.

RESULTS

Test for Helicity of the Modified Pro-a2(I) Chains—Isolated a2(I) chains reassociate slowly at 16 °C to form a helical structure with a Tm of 20–24 °C compared to a Tm of 38–40 °C for type I collagen (13, 14). Our previous studies showed that a-chains could be obtained from the modified pro-a2(I) chains by pepsin digestion under stringent conditions at 4 °C, suggesting that a triple helix was formed at this temperature. We compared the stability of these helical structures to those of normal collagens, using resistance to trypsin proteolysis as an index of helical structure. In this assay, collagens are exposed to increasing temperatures, are rapidly treated with a large excess of trypsin at neutral pH, and then are analyzed by SDS-PAGE. When the melting temperature is reached, the a-chains become susceptible to proteolysis. Normal type I collagen was isolated by pepsin digestion of the P33 fraction of medium from nontransformed SHE fibroblasts. It showed melting between 38 and 40 °C (Fig. 1A), which is typical for type I collagen from various sources (10–13). As reported previously (5), the pepsin-treated P33 fraction from the medium of NQT-SHE cells contains, in addition to the N33a chain, a small proportion of normal type V collagen that is composed of a1(V) and a2(V) chains. These served as an internal control (Fig. 1B). Melting of type V collagen occurred between 36 and 38 °C, which is similar to the melting temperature ranges reported previously (18, 19). The band just above N53a is a pepsin-resistant fragment of a noncollagenous protein which was not degraded further by trypsin. The N33a chain was degraded after it was equilibrated at 30 °C (Fig. 1B), so the starting temperature was decreased to 5 °C. Both of the N-collagen a-chains were degraded after exposure to 5 °C (Fig. 1, C and D), while type V collagen was completely resistant up to 30 °C (Fig. 1D).

Effect of Inhibition of Proline Hydroxylation on N-Collagen Secretion—The helical form of procollagen I is preferentially

![Fig. 1. Trypsin-resistance assay for collagen helix stability.](Image)
secreted and the stability of the helix is dependent on the presence of hydroxyproline (4). Therefore, inhibition of prolyl hydroxylase, either by omitting ascorbate or adding an iron chelator such as $\alpha\alpha'$-dipyridyl, leads to inhibition of secretion (4, 20–22). Lyasyl hydroxylase exhibits the same requirements as prolyl hydroxylase, but the absence of hydroxylysine in procollagen does not affect its secretion (23). We measured the synthesis and secretion of procollagens in SHE and transformed NQT-SHE fibroblasts in the presence and absence of ascorbate. SHE fibroblasts synthesize mainly type I procollagen and its rate of secretion was inhibited by omission of ascorbate (Fig. 2A). Secretion of type I procollagen from BHK cells, an established line of normal Syrian hamster fibroblasts, was affected similarly by omitting ascorbate (data not shown). Secretion of procollagens from NQT-SHE was not affected by omitting ascorbate (Fig. 2B) and the time course for secretion was similar to that of hydroxylated procollagen I from SHE cells. These results represent mainly secretion of the N-collagens, which comprise more than 90% of the procollagens secreted by NQT-SHE cells (5). It should be noted that secretion is expressed as a percentage of the total procollagen produced that was found in the medium, rather than as an absolute rate in terms of molecules of procollagen secreted.

The data in Table I show the extent of inhibition of proline hydroxylation obtained by omitting ascorbate or adding the iron chelator $\alpha\alpha'$-dipyridyl and the effects of these treatments on procollagen secretion (Table I). Proline hydroxylation in both SHE and NQT-SHE cells was inhibited about 85% by omitting ascorbate from cultures and almost completely by adding $\alpha\alpha'$-dipyridyl. The rate of secretion of procollagen from SHE cells was significantly inhibited when hydroxylation was inhibited. As shown in previous reports (21, 22), however, when hydroxylation is completely inhibited by the chelator, there is a slow rate of secretion of unhydroxylated procollagen. Secretion of procollagen from NQT-SHE cells was unaffected when proline hydroxylation was inhibited by either method.

SDS-PAGE analysis of procollagens secreted from NQT-SHE fibroblasts labeled with $[^{14}C]$proline in the presence or absence of ascorbate produced similar results. Approximately equivalent amounts of N33 and N50 chains were present that migrated slightly more slowly than pro-$\alpha$-I chains (data not shown). Pepsin digestion of the unhydroxylated N-collagens yielded $\alpha$ chains that migrated more slowly than the normal $\alpha$2(1) chain (data not shown). These results suggested that the modified behavior of the N-collagens might not be due to hyper-hydroxylation, but since the differences in migration between normal and modified $\alpha$2(I) chains are small, this possibility was examined further by analyzing peptides derived from the N-collagens by V8 protease digestion.

**Peptide Maps of Hydroxylated and Unhydroxylated Collagen Chains**—Our previous results showed that the altered behavior of the N-collagens was reflected and amplified in two peptides (V8-1 and V8-2) derived by V8 protease digestion (5). Conditions used for SDS-PAGE in the present experiments enhanced the differences in the migration of the V8-1 and -2 peptides from secreted hydroxylated $\alpha$-chains (Fig. 3). Normal $\alpha$2(I) chains from SHE cells were digested in several lanes (A, lanes 1, 4, and 11; B, lane 2) in order to insure an adequate basis for comparison regardless of gel imperfections. The peptide maps from the hydroxylated $\alpha$2(I) chains secreted by BHK and SHE cells were identical (data not shown). The peptides from N33$\alpha$ (A, lane 2) and N50$\alpha$ (A, lane 3; B, lane 1) migrated more slowly than the analogous peptides from the normal $\alpha$2(I) chain. In addition, the V8-1 and -2 peptides from N50$\alpha$ migrated more slowly than those from N33$\alpha$ (A, lane 3 versus 2). The diffuseness of the V8-1 and -2 bands from N33$\alpha$ and N50$\alpha$ might be due to heterogeneity in the extent of modification. As reported previously (5), a number of peptides that appeared below the 30-kDa marker showed almost identical migration whether obtained from the normal or modified $\alpha$2(I) chains. Based on a comparison to collagen CB peptide markers (data not shown), which migrate more slowly than globular polypeptides of the same size, V8-1 and -2 from the normal $\alpha$2(I) chain are approximately 38 and 25 kDa, respectively.

To determine if the modified behavior of N-collagens was due to post-translational modification and might have resulted from hyper-hydroxylation of nonhelical chains, we also compared the V8 protease peptide maps from hydroxylated...
and unhydroxylated α-chains derived from intracellular and medium procollagens (Fig. 3). The secreted unhydroxylated N33α chain gave rise to V8-1 and -2 peptides (A, lane 6; B, lane 4) that migrated as slowly as the hydroxylated N33α peptides (A, lane 2), i.e. slower than normal α2(I) V8-1 and -2. Comparison with the normal hydroxylated α2(I) peptides can be made by examining; A, lane 6 (unhydroxylated N33α) versus lane 4 (hydroxylated α2), and, B, lane 4 (unhydroxylated N33α) versus lane 2 (hydroxylated α2). Unhydroxylated V8-1 and -2 peptides from N50α (B, lane 5), however, migrated identically to those from unhydroxylated N33α (A, lane 6; B, lane 4). Surprisingly, V8-1 and -2 peptides from unhydroxylated α2(I) chains secreted by normal BHK cells (A, lane 5) also migrated more slowly than the normal hydroxylated peptides. This effect was specific to the hydroxylated α2(I) chains derived from procollagen secreted by BHK cells, migrated slightly more slowly than the peptide from the hydroxylated chain (lane 9 versus lane 8), analogous to the behavior of the V8-1 and -2 peptides from this polypeptide.

Because both the V8-1 and -2 and CB3,5 peptides from Nα-chains migrated more slowly than their normal counterparts, it seemed likely that these peptides were derived from the same region of the Nα-chains. This possibility was examined by excising the CB peptides from the same gel used to produce the fluorogram shown in Fig. 4, and digesting them with V8 protease (Fig. 5). Two concentrations of V8 protease were used to determine whether V8-2 was a product of V8-1, and

V8-1 and -2 peptides from intracellular N33α chains, whether hydroxylated (A, lane 10) or not (A, lane 8), migrated similarly to the peptides from intracellular α2(I) chains produced by normal cells (hydroxylated; A, lane 9; unhydroxylated, A, lane 7; B, lane 3). The slight diffuseness of the V8-2 band from intracellular N33α (A, lanes 8 and 10) suggests that a low level of modification occurred in this region prior to secretion, but the majority of the band appeared to be unmodified.

**Location of the Modification Within the α2(I) Chain**—Our previous results suggested that there were no differences in the CB peptides derived from normal α2(I) and Nα-chains (5). When electrophoretic conditions were modified, however, the CB3,5 peptides derived from N33α and N50α migrated more slowly than the normal α2(I) CB3,5 peptide (Fig. 4, lanes 1–6). There were no differences in the migration of the CB4 peptides. In addition, there appeared to be increased cleavage of CB3,5 to CB3 and CB5, which migrate to positions just above the CB4 peptide. As in the case of the V8 peptides, CB3,5 from unhydroxylated N33α retained its modified behavior (lane 10). The CB3,5 peptide from unhydroxylated α2(I) chains derived from procollagen secreted by BHK cells, migrated slightly more slowly than the peptide from the hydroxylated chain (lane 9 versus lane 8), analogous to the behavior of the V8-1 and -2 peptides from this polypeptide. However, the differences between the CB3,5 peptides from the N-collagen α-chains compared to the normal peptides were not as great as the differences observed with the V8-1 and V8-2 peptides, which are smaller and resolved better on gels.
Secretion-Incorporation of the proline analog cis-hydroxyproline leads to decreased stability of the helical structure and inhibition of procollagen secretion (4, 22, 25). This represents a specific effect on collagen production. Identical results were obtained with both radioactive precursors. There also may be increased intracellular degradation of the denatured chains (26). The effect of the analog on secretion of helical type I procollagen from normal SHE and BHK cells was determined (Table II). With SHE cells, secretion was inhibited 21% at 5 mM and 75% at 15 mM. A similar concentration range was effective for BHK cells, with 46.7% inhibition at 10 mM. In both cases, there was little effect on the relative rate of collagen production, which reflects the sum of synthesis and degradation.

Concentrations of cis-hydroxyproline up to 10 mM were tested for effects on NQT-SHE cells (Fig. 7). Cells were double labeled with $[^3H]$proline and $[^3]C$glycine in order to eliminate possible effects of the analog on proline transport or aminoclaylation of prolyl tRNA. There was relatively little effect on procollagen secretion (Fig. 7, bottom), but the relative rate of production was inhibited by 45% at 10 mM (Fig. 7, bottom). This represents a specific effect on collagen production. Identical results were obtained with both radioactive precursors. The small amount of inhibition of secretion that was observed in the presence of 6–10 mM cis-hydroxyproline might be due to inhibition of type V collagen secretion. This possibility was supported by the finding that type V collagen was not present...
produced by NQT-SHE cells are unusual, based on the current treatments. The relative rate of collagen production (---) and the extent of its inhibition by cis-hypro (-- -) are shown in the top panel and the percentage of secretion is shown in the bottom panel. Results are the averages from duplicate cultures.

in the medium of cells treated with cis-hydroxyproline, as determined by SDS-PAGE, although intact N33 and N50 were present (data not shown).

To determine whether the decreased rate of procollagen production induced by cis-hydroxyproline was due to an effect on synthesis or degradation, a pulse-chase experiment was carried out. NQT-SHE cells were labeled for 1 h with radioactive proline in the presence or absence of the analog. During this interval, relatively little procollagen is secreted. The analog was removed and radioactivity was chased with 50 mM proline. The radioactivity remaining in procollagen in the cell and medium fractions was measured at various chase intervals (Fig. 8). The total pool of N-collagens that were pulse-labeled in the absence of the analog was quite stable, while N-collagens pulse-labeled in its presence were degraded during the chase (Fig. 8 A). There was essentially no degradation of noncollagenous proteins in either the control or cis-hydroxyproline-treated cells (data not shown), so the effect on the N-collagens was quite specific. Analysis of the cell and medium compartments separately showed that the proportions of the radioactive N-collagens that were secreted during the chase period were almost identical in control and cis-hydroxyproline-treated cultures (Fig. 8 B). In control cultures, there was a gradual loss of procollagen from the cell fraction that could be accounted for by the amount secreted (Fig. 8C, solid line), but intracellular procollagen from the cis-hydroxyproline-treated cultures was degraded (Fig. 8C, dashed line).

**DISCUSSION**

The secretion properties of the pro-a2(I)-related chains produced by NQT-SHE cells are unusual, based on the current concept for the regulation of procollagen secretion. It was previously shown that a triple helix consisting of a2(I) chains is unstable (13, 14). Our results suggest that the helical forms of the N-collagens that were obtained by pepsin digestion at 4 °C are even more unstable, so that it is unlikely that they would assume a helical configuration under culture conditions. This conclusion is supported by the observations that inhibition of proline hydroxylation or incorporation of cis-hydroxyproline into procollagen, which cause disruption of the procollagen helical structure (4, 22, 25) inhibited secretion of type I procollagen from control SHE and BHK cells but

**FIG. 7. Effect of cis-hydroxyproline (cis-hypro) on the relative rate of procollagen production and secretion by NQT-SHE fibroblasts.** The procedures used were similar to those described in the legend to Table II except that 10 μCi of [5-3H]proline and 2 μCi of [1-14C]glycine were added to each dish and radioactivity from tritium (○) or 14C (□) was analyzed by double label counting techniques. The relative rate of collagen production (---) and the extent of its inhibition by cis-hypro (-- -) are shown in the top panel and the percentage of secretion is shown in the bottom panel. Results are the averages from duplicate cultures.

**FIG. 8. Pulse-chase analysis of N-collagens radiolabeled in the absence or presence of cis-hydroxyproline.** NQT-SHE cells in 60-mm dishes were preincubated in 1 ml of serum-free medium as described in the legend to Table II, except that 10 mM cis-hydroxyproline (cis-hypro) was either absent (○) or present (□) and cells were labeled with [5-3H]proline (20 μCi) for 1 h. Duplicate samples of the cell fractions were harvested for analysis immediately (zero time samples). The media from the remaining dishes were replaced with fresh serum-free medium containing 0.1 mM each of &amino-propionitrile and ascorbate, and 50 mM unlabeled proline and incubation at 37 °C was continued for 30, 60, or 120 min (chase period). The cell and medium fractions were separated and analyzed for the amount of radioactive collagen remaining, as described under "Experimental Procedures." A, the percentage of pulse-labeled collagen remaining in the cell plus medium fractions during the chase period. The amount of radioactive collagen in the cells at zero time is considered as 100%. B, the percentage of the pulse-labeled collagen appearing in the medium. C, the percentage of pulse-labeled collagen remaining in the cell fraction. Radioactivity in collagen (disintegrations/min × 10^6) per dish) in the zero time samples were: control, 95.7; plus cis-hypro, 50.7.
failed to affect secretion of the modified pro-α2(I) chains. In spite of nonhelicity of the N-collagens, the time course for their secretion, whether hydroxylated or not, was similar to that for secretion of hydroxylated helical type I procollagen from normal SHE or BHK fibroblasts.

Although the helical domain of type I procollagen is resistant to proteolysis, denatured chains are susceptible. Earlier studies (15, 16) suggested that pro-α2(I) chains that could not be incorporated into a stable heterotrimer were degraded, but we found almost no degradation of the modified chains in a pulse-chase experiment. The intracellular degradation of procollagen containing cis-hydroxyproline that was observed in some cell types has been attributed to the disruption of the helical structure (4, 26). Pulse-chase experiments with NQT-SHE cells revealed that the analog induced rapid intracellular degradation of the N-collagens, although they already were in denatured form. Approximately 60% of the N-collagens escaped degradation and were secreted, probably because during the pulsing period they had passed through the site where degradation occurs. In normal cells that synthesize both subunits of type I procollagen, failure to form a stable triple helix because of incorporation of cis-hydroxyproline into the chains may be the direct cause for inhibition of secretion, but degradation most likely results from recognition of the denatured chains as abnormal proteins. Incorporation of amino acid analogs into proteins leads to enhanced degradation of the resulting abnormal molecules (27) and some abnormal cytosolic proteins, such as globin, are degraded after conjugation with ubiquitin (27, 28). Degradation of membrane or luminal proteins in the rough endoplasmic reticulum has been described and it appears to result from the failure to assemble oligomeric structures (29). It is not yet clear whether secreted proteins containing amino acid analogs are degraded via the endoplasmic reticulum system or whether ubiquitinylation might be involved.

The modification of the N-collagens is localized within the region spanned by the cyanogen bromide peptide CB3,5, which covers approximately 60% of the polypeptide and extends to the carboxyl terminus. Two V8 protease-derived peptides, V8-1 and -2, that exhibit modified electrophoretic behavior are from the same region and appear to be independent cleavage products. Hydroxylation of Pro and Lys residues and subsequent glycosylation of Hyl residues occur rapidly in the rough endoplasmic reticulum (4). Therefore, hyper-hydroxylation of nonhelical procollagen chains should be found in both intracellular and secreted molecules. This does occur in the case of the mutated thermally unstable type I procollagen produced by fibroblasts from osteogenesis imperfecta patients (11). In the case of the N33a chain, however, the V8-1 and -2 peptides derived from the cell fraction were not modified. This result suggests that the modification was not due to hyper-hydroxylation. It also rules out the possibility of a mutation in the pro-α2(I) gene that would result in an amino acid change which directly alters electrophoretic mobility. It also is unlikely that a mutation in NQT-SHE cells led to substitution of an amino acid that became susceptible to a post-translational modification. This conclusion is based on the results obtained for normal BHK cells which showed that the hydroxylation-independent modification can occur in the absence of carcinoenogen-induced transformation. The V8-1 and -2 and CB3,5 peptides from secreted, but not intracellular, unhydroxylated pro-α2(I) chains produced by BHK cells exhibited retarded migration compared to their hydroxylated counterparts. Further evidence that modification of the N-collagens occurs independently of hydroxylation, were the observations that the CB3,5 and the V8-1 and -2 peptides from unhydroxylated N33a migrated similarly to the V8-1 and -2 peptides from hydroxylated N33a, i.e. slower than normal. The remaining V8 peptides migrated similarly to, or slightly faster than, their hydroxylated counterparts, as would be expected for unhydroxylated collagenous peptides (11), pointing up the unique behavior of the V8-1 and -2 peptides. The N50α chain, however, appears to be hyper-hydroxylated, in addition to containing the hydroxylation-independent modification.

In summary, this study has revealed several features of procollagen biochemistry that do not adhere to previously held concepts, such as the finding that pro-α2(I) chains can be secreted in nonhelical form in the absence of the other type I subunit, pro-α1(I). Our results also suggest that when type I procollagen chains remain nonhelical during cellular translocation, either because of inhibition of hydroxylation in normal cells that produce both the pro-α1 and pro-α2 subunits, or because the pro-α1(I) subunit is not synthesized, as in NQT-SHE cells, then the pro-α2(I) chains are post-translationally modified before secretion. Post-translational modifications in the collagenous domain other than hydroxylation of Pro and Lys residues and glycosylation of Hyl residues have not been reported, so that the modification we have described is a novel reaction that remains to be characterized. While it is possible that the modification is merely a consequence of non-helicity and has no biological significance, it is equally possible that its presence is related to the ability of the nonhelical N-collagens to escape degradation. Further studies will be required to resolve this question. Finally, our results show that cis-hydroxyproline induces degradation of nonhelical procollagen chains, contrary to the previously held concept that analog-induced degradation occurs because the analog destabilizes the triple helix and thus allows access of the denatured chains to degradative processes.

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