Ascorbate Differentially Regulates Elastin and Collagen Biosynthesis in Vascular Smooth Muscle Cells and Skin Fibroblasts by Pretranslational Mechanisms*

(Received for publication, February 29, 1996, and in revised form, October 7, 1996)

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Ascorbate contributes to several metabolic processes including efficient hydroxylation of hydroxyproline in elastin, collagen, and proteins with collagenous domains, yet hydroxyproline in elastin has no known function. Prolyl hydroxylation is essential for efficient collagen production; in contrast, ascorbate has been shown to decrease elastin accumulation in vitro and to alter morphology of elastic tissues in vivo. Ascorbate doses that maximally stimulated collagen production (10–200 μM) antagonized elastin biosynthesis in vascular smooth muscle cells and skin fibroblasts, depending on a combination of dose and exposure time. Diminished elastin production paralleled reduced elastin mRNA levels, while collagen I and III mRNAs levels increased. We compared the stability of mRNAs for elastin and collagen I with a constitutive gene after ascorbate supplementation or withdrawal. Ascorbate decreased elastin mRNA stability, while collagen I mRNA was stabilized to a much greater extent. Ascorbate withdrawal decreased collagen I mRNA stability markedly (4.9-fold), while elastin mRNA became more stable. Transcription of elastin was reduced 72% by ascorbate exposure. Differential effects of ascorbic acid on collagen I and elastin mRNA abundance result from the combined, marked stabilization of collagen mRNA, the lesser stability of elastin mRNA, and the significant repression of elastin gene transcription.

Ascorbate, along with ferrous ion and α-ketoglutarate, is a cofactor for the enzymatic activity of prolyl hydroxylase, a heteromer that hydroxylates prolyl residues in procollagen, elastin, and other proteins with collagenous domains prior to triple helix formation (1–6). Ascorbate in low concentrations is essential for production of collagen, since a minimum of 35% of the prolyl residues in collagen need to be hydroxylated for the collagen molecule to maintain its triple-helical conformation at physiologic temperatures (3). Ascorbate is also a cofactor for lysyl hydroxylase (7). Further modification of hydroxylysine has key effects on collagen fiber organization (8).

In addition to its direct, rapid effect on hydroxylatation, ascorbate, at levels approaching 50 μM, has been found to cause a 6-fold increase in the rate constant for procollagen secretion (9), as well as an increase in collagen gene transcription and collagen mRNA levels in various cell strains (9–17). This suggests that ascorbate action not only involves hydroxylation and stabilization of the triple helix, it also involves direct or indirect effects on gene expression and protein secretion. The increase of type I collagen production in cells cultured in the presence of ascorbic acid is well known (5, 15, 18–23) and has been investigated extensively; however, the effects of ascorbate on other extracellular matrix molecules are still poorly understood. Several studies have shown that vitamin C exerts a negative effect on elastin accumulation (5, 24–26). Although hydroxyproline is a normal, minor constituent of insoluble elastin, it has been suggested that ascorbate might impair elastin production by overhydroxylation of its prolyl residues (24, 29, 30). However, elastin secretion is hydroxylation-independent (31).

The aims of our study were to investigate, in two different cell culture models: (a) the time- and dose-dependent effect of ascorbate on type I collagen and elastin production and mRNA expression, and (b) evidence for these effects being due to a pre- and/or posttranslational mechanism of regulation. The possible effect of the redox properties of this vitamin on connective tissue metabolism is discussed.

MATERIALS AND METHODS

Cell Culture—Pig aortic and pulmonary artery smooth muscle cells and pig skin fibroblasts were obtained from newborn to 14-day-old domestic pigs sacrificed by anesthesia and exsanguination. Tissues were removed and placed immediately on ice in transport medium consisting of Dulbecco’s modified Eagle’s medium (DMEM) containing 5% fetal calf serum (FCS; HyClone Laboratories, Logan, UT), 1000 units/ml penicillin, 1000 μg/ml streptomycin, 2.5 μM fungizone (Life Technologies, Inc.), 0.03% glutamine, 1 mM sodium pyruvate, and 0.1 mM nonessential amino acids (Life Technologies, Inc.). Using sterile technique, descending aortas from several 1-day-old pigs were stripped of the external, adventitial layer and dissected into four serial segments, comparable in length, designated as A, B, C, and D as described previously (32). Tissues were pooled according to segments, finely minced, and incubated with 200 units of crude collagenase (type IA, Sigma) in transport medium for 4–6 h at 37 °C. Digested tissue was washed to remove excess collagenase, and cells were allowed to migrate

* This work was supported by Grant GM37387 from the NIGMS, National Institutes of Health and by the Department of Veterans Affairs. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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¶ Printed in U.S.A.

1 The abbreviations used are: DMEM, Dulbecco’s modified Eagle’s medium; FCS, fetal calf serum; ELISA, enzyme-linked immunosorbent assay; TE, tris-O-acetic acid; PBS, phosphate-buffered saline; mRNA, elastin mRNA; kb, kilobase(s); SMC, smooth muscle cell(s).
from explants in plastic tissue culture dishes (Corning, Marietta, GA) in DMEM, 20% FCS and antibiotics and were maintained in 5% CO2, 95% humidified air at 37°C. Pig pulmonary arteries (divided in inner and outer medial layers), pig skin, and human skin biopsies were cultivated by outgrowth as described (33).

The pro-α1(1)I collagen, type I procollagen, and tropoelastin (0.2% Ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin) were used to coat the plates at a 1:2 ratio in 15 m g/ml ascorbate, and pig TE (0.17–22 ng) was used for constructing a standard curve. Native type I collagen was used to evaluate both type I procollagen and tropoelastin (TE).

Quantitation of Soluble Collagen and Elastin—A standard ELISA was used to evaluate both type I procollagen and tropoelastin (TE) production in media samples from the same cell populations (35, 36). Type I procollagen production was measured in 48-h media using rabbit antiserum against the triple-helical portion of native, porcine, type I collagen. The IgG fraction from this antiserum showed no cross-reactivity with porcine type III or type V collagens (35). Native type I collagen was used for coating the plates and as the competing antigen in order to generate a standard curve.

TE production in pig smooth muscle cells and pig fibroblasts was evaluated in 48-h medium using rabbit antiserum to pig α1-elastin at a 1:2000 dilution. Pig α1-elastin (40 ng/ml) was used as the adsorbing antigen, and pig TE (0.17–22 ng) was used for constructing a standard curve. The same conditions were used for elastin quantitation in cultured human fibroblasts, except that the coating antigen was human α1-elastin. Data for both type I collagen and TE production were calculated as the ratio of each transcript signal relative to the constitutive, 1B15 signal, or to each other.

RESULTS

Ascorbate Reduces Elastin mRNA

Ascorbate reduces elastin mRNA by 18–36% of human elastin (44); (c) pHII-I, a 1.9-kb collagen type III cDNA insert in the rat I site of pGEM-4Z (Promega, Madison, WI) corresponding to the α1 chain of type III collagen; (d) tGMM71, a 1.6-kb fibronectin cDNA fragment in the EcoRI site of pBR322 corresponding to part of the fibrin-binding domain and (e) pG4MT4HB, a 550-base pair rat stromelysin-1 cDNA insert in the BamHI and HindIII sites of pGEM-4Z, corresponding to a portion of stromelysin located 440 base pairs from its 3′ end (47). The probes were labeled with [32P]dCTP (DuPont NEN) by random priming (48, 49) to a specific activity of 0.5–1.5 × 108 dpm/μg of DNA. The hybridization mixture, containing the labeled probe (30,000 cpm/μg), 100 μg/ml salmon sperm DNA/μg, 100 μg/ml yeast tRNA (Sigma), 30–50% formamide (Fisher), 12.5% dextran sulfate (Pharmacia Biotech Inc.), 0.1% dithiothreitol and 30% formamide, and then in decreasing concentrations of SSC to 0.1 × SSC at 45°C followed by dehydration. The slides were dipped in Kodak NTB2 emulsion, boxed for 7 days at 4°C, and then developed in Kodak D19 developer and fixed at 15°C. Cells were stained with hematoxylin and eosin, observed in bright and dark field with an Olympus BH-2 photomicroscope and photographed with a Panatomic film.

Effects of Ascorbate on Collagen and Elastin Production—Preconfluent monolayers of porcine vascular smooth muscle cells (SMC) were continuously exposed to 50 μg/ml ascorbate, a
Ascorbate reduces elastin mRNA

**FIG. 1.** Type I collagen production and cell proliferation are stimulated in pig aortic smooth muscle cells at low and high cell density. Second passage, preconfluent smooth muscle cells from a 1-day-old pig were supplemented with daily doses of 50 μg/ml ascorbate. Collagen production over 48-h intervals was evaluated by ELISA of the culture medium. Production data are expressed as molecular equivalents/cell h ± S.E. of collagen type I produced by triplicate cultures grown in the presence (open bars) or absence (solid bars) of ascorbate. Open and closed circles represent cell numbers from ascorbate-treated and ascorbate-deficient cultures, respectively. The arrow indicates the time at which cells reached visual confluence.

Ascorbate concentration that maximally stimulates collagen production, with addition of fresh ascorbate every 24 h. Less frequent addition did not produce consistent effects (data not shown). As shown in Fig. 1, the expected stimulatory effect on collagen I production in these cultures was independent of cell density. From the time of treatment (3 days before confluence) until the termination of the experiment, collagen production was amplified from 2- to 8-fold by treatment with vitamin C. Under scurvy conditions, we observed a small, significant rise in collagen production in postconfluent (>72 h) cultures. Since smooth muscle cells continue to overgrow after reaching confluence, both ascorbate-supplemented and deficient cultures showed a continuous increase in cell number. Ascorbate-treated cultures grew at a slightly faster rate. In contrast to the positive effects on collagen production, tropoelastin production was markedly diminished by ascorbate treatment (Fig. 2). As shown previously (36), tropoelastin production under control conditions did not approach a maximum until cells reached confluence (72 h). Thus, the most significant inhibition by ascorbate was seen in postconfluent SMC cultures. Tropoelastin production on different days of culture ranged from 30% to 50% of untreated cultures.

As suggested from the foregoing data, ascorbate effects depend on the differentiation state of the target cell. In one experiment, we compared ascorbate inhibition in thoracic versus abdominal aortic SMC. Previous experiments have shown marked, site-specific differences in basal production of tropoelastin in this tissue (32, 33). Fig. 3 illustrates the 2- to 5-fold higher basal production levels in SMC from thoracic versus abdominal aortic segments of the newborn pig. When SMC from each of these segments were treated with ascorbate, the relative inhibition in the thoracic segments was far greater than in the abdominal segments.

Ascorbate inhibition of tropoelastin production showed a dose-dependent relationship (Fig. 4). Inhibition was observed in some experiments at doses as low as 0.5 μg/ml ascorbate, and the degree of inhibition was significant at 5–10 μg/ml. Effects of ascorbate were not immediate. Fig. 5 illustrates the time and dose-dependent effects of ascorbate on a different cell population, human skin fibroblasts. In this experiment, it was observed that daily addition of at least 60 μg/ml ascorbate was required to obtain significant inhibition, and exposure to the vitamin for 3 days was necessary at this dose. At higher doses (100 μg/ml) inhibition was more immediate and more extensive. In many subsequent experiments, we used a protocol in which ascorbate was added at lower, divided doses in an effort to maintain a more constant, biologically active concentration. This treatment regime produced the most consistent and effective inhibition of TE production.

To help confirm the specificity of the ascorbate effect, confluent, thoracic SMC were incubated with another reducing agent, glutathione (GSH; 0.28–3.0 mM), and an inactive form of ascorbate, dehydroascorbic acid (0.28 mM). At levels up to 1.0 mM, glutathione did not reduce TE production, while at a 10-fold molar excess relative to ascorbate (3.0 mM), there was a partial diminution of TE production (Fig. 6). The latter effect was attributable to cytotoxicity of the compound (data not shown). Dehydroascorbate treatment showed no significant effect on TE production, while ascorbate at an equivalent concentration produced a ~60% reduction in TE production.

Ascorbate reduces elastin production by pretranslational mechanisms—Messenger RNA levels for collagen I and elastin

**FIG. 2.** Ascorbate inhibits tropoelastin production particularly in postconfluent pig aortic smooth muscle cells. Preconfluent SMC at second passage were exposed daily to 50 μg/ml ascorbate, as described in Fig. 1. Tropoelastin production was evaluated by ELISA in medium accumulated over 48-h intervals and expressed as molecular equivalents/cell h ± S.E. Open and closed bars represent cells grown in the presence or absence of ascorbate, respectively. Cell number data are identical to those of Fig. 1.

**FIG. 3.** Segmental variation in the effect of ascorbate on tropoelastin production. Pig smooth muscle cells derived from thoracic (A) and abdominal (D) segments of a newborn porcine aorta were maintained in 50 μg/ml/day ascorbate. Culture medium was changed every 3 days. At confluence media were changed and harvested 48 h later for evaluation of elastin production, which is expressed as molecular equivalent/cell h ± S.E. Solid bars, TE production − ascorbate; open bars, TE production + ascorbate; open circles, cell number + ascorbate; closed circles, cell number − ascorbate.
were evaluated subjectively by in situ hybridization of skin fibroblasts grown in multwell culture slides in the presence of varying concentrations of ascorbate (Fig. 7). Under these culture conditions, a 24-h exposure was sufficient to alter markedly the relative abundance of these two transcripts. In Fig. 7, the upper panel shows that ascorbate was able to cause dose-dependent increases in collagen I transcript levels, while the lower panel shows a dose-dependent inhibition of elastin mRNA under the same culture conditions. In sister cultures, ascorbate was effective in increasing COL3A1 mRNA levels, while no remarkable changes were seen in mRNA levels for fibronectin and stromelysin (data not shown). Reduction of mRNAE by increasing ascorbate dose was quantified by dot blot hybridization and Northern blot analysis; elastin mRNAE steadystate levelsin the presence of 10 and 50 \( \mu \text{g/ml} \) ascorbate were 30–40% of that seen without ascorbate. Fig. 8 shows the kinetics of mRNAE reduction when porcine pulmonary aorta SMC were exposed to two daily additions of 50 \( \mu \text{g/ml} \) ascorbate over a 48-h period. The data, normalized to the expression of a constitutivetranscript (cytochrome oxidase II; Refs. 53 and 54), showed that elastin mRNA under these circumstances was reduced to less than 20% of control values.

Since several factors modulate elastin transcript levels by altering mRNA stability (55), we evaluated the stabilities of elastin and collagen I mRNAs under conditions of continuous ascorbate exposure and upon withdrawal of ascorbate. Smooth muscle cultures that had been previously treated with ascorbate (2 \( \times \) 50 \( \mu \text{g/ml} \) day) were changed to medium lacking ascorbate and containing actinomycin D to block transcription. When ascorbate was removed from the cultures, there was an increase in the apparent \( t_1/2 \) of mRNAE from approximately 19 h to 33 h (Fig. 9, panel A). This stability difference was selective, since mRNAE was less stable than a constitutive gene, cyclophilin, only in the presence of ascorbate (Fig. 9, panel B).
 autoradiographic images of mRNAE were normalized to the intensity of hybridization with an elastin cDNA probe. Densitometric analysis of transcripts during the first 12 h after ascorbate withdrawal.

Conversely, COL1A2 transcripts were markedly destabilized by ascorbate withdrawal (t1/2 ≈ 7 h versus 32 h; Fig. 9, panel C). Because of the inverse behavior of the two transcripts, ascorbate depletion caused rapid rise in the ratio of ELN to COL1A2 transcripts during the first 12 h after ascorbate withdrawal (Fig. 9, panel D, open circles), while in the presence of ascorbate the proportion of mRNAE to COL1A2 mRNA progressively declined (Fig. 9, panel D, solid squares).

These studies using a transcription inhibitor suggested that reduced mRNAE stability was insufficient to account fully for reduced mRNAE levels in ascorbate-treated cells, while changes in COL1A2 transcript stability were correlated with the biosynthesis of elastin and mRNAE. The requirement for ascorbic acid to maintain elastin biosynthesis, was actually an antagonist of elastin accumulation in the biosynthesis of elastin. Using proline analogs or hypoxic conditions, these investigators demonstrated that prolyl hydroxylation was not required for the biosynthesis and secretion of elastin, suggesting that hydroxyproline either played a different role in elastin or was produced as a byproduct of the coincident synthesis of collagen and elastin by many types of cells (30, 31, 60). In contrast, the findings of de Clerck and Jones (5) and Scott-Burden et al. (28) suggested that ascorbate, at concentrations that maximally stimulated collagen biosynthesis, was actually an antagonist of elastin accumula-

**DISCUSSION**

The requirement for ascorbic acid to maintain connective tissue integrity was observed centuries ago by Lind (56). Subsequently, ascorbate was found to play a role in several physiologic systems (57, 58). With modern appreciation of the primary structure of collagen came the understanding that hydroxyproline played a crucial role in stabilizing the collagen triple helix. In vitro and in vivo studies indicated that ascorbic acid played a role in the generation of this important posttranslational modification, and detailed biochemical investigation revealed the precise role of ascorbate during the transfer of molecular oxygen to prolyl (and lysyl) residues (59). The observation that elastin also contained a significant amount of hydroxyproline led a number of investigators to examine its role in the biosynthesis of elastin. Using proline analogs or hypoxic culture conditions, these investigators demonstrated that prolyl hydroxylation was not required for the biosynthesis and

**FIG. 8.** Kinetics of mRNAE reduction by ascorbate. Smooth muscle cells from the pulmonary aorta of a 6-month-old pig were grown in 100-mm tissue culture dishes. At confluence cells were fed with DMEM, 5% newborn calf serum and supplemented with three daily additions of 50 µg/ml ascorbate (solid symbols) or nothing (open symbols). RNA was isolated from cells at indicated times and identified by Northern blot hybridization with an elastin cDNA probe. Densitometric analysis of autoradiographic images of mRNAE were normalized to the intensity of the constitutive probe, cytochrome oxidase II.

**FIG. 9.** Effect of ascorbate withdrawal on stabilities of elastin and collagen mRNA. Cultures of porcine SMC were pretreated for 24 h with 2 doses of 50 µg/ml ascorbate. Actinomycin D (7 µg/ml) was added at time = 0, and ascorbate was maintained or removed. Solid squares, ascorbate maintenance; open circles, ascorbate withdrawal. mRNA levels were quantified by scanning densitometry of Northern blots hybridized to ELN, COL1A2, and cyclophilin probes. Panel A, effect of ascorbate withdrawal on elastin mRNA stability. Hybridization signals for elastin mRNA in the presence and absence are expressed in relative optical density units on a logarithmic scale. Data are normalized for differences in input RNA. Linear regression curves with and without ascorbate present showed differing apparent decay rates, although the scatter of the data yielded slopes that were not statistically different (0.1 > p > 0.05). The estimated t1/2 was ~32 h in the absence and ~19 h in the presence of ascorbate. Panel B, elastin mRNA stability normalized to a constitutive gene, cyclophilin. To correct for possible loading differences and for general effects, signals for mRNAE were related those of cyclophilin and normalized to unity at the start of actinomycin D addition and ascorbate withdrawal (0 h). Data are on a logarithmic scale. Panel C, effect of ascorbate withdrawal on collagen mRNA stability. COL1A2 levels were normalized to the constitutive gene, cyclophilin, and plotted on a logarithmic scale. Collagen mRNA was markedly destabilized by ascorbate withdrawal. Panel D, elastin mRNA is relatively more stable than collagen I mRNA upon ascorbate withdrawal. Experimental data compare the ratio of elastin mRNA to collagen I β2 chain mRNA as a function of time after addition of actinomycin D (t = 0) and maintenance or withdrawal of ascorbate. Elastin mRNA was much less sensitive to ascorbate withdrawal than collagen I, thus maintaining a higher ratio. In the continued presence of ascorbate, mRNAE was slightly less stable than collagen I mRNA, causing a fall in the ratio.
Ascorbate Reduces Elastin mRNA

Effects of vitamin C on elastin production in cell culture depend on both dose and time of exposure. The fact that lower doses of ascorbate appeared to require longer exposure times may be related to the rapid oxidation of ascorbic acid under standard culture conditions. Although biological effects could be reproducibly observed with daily doses of ascorbate, more effective or dramatic effects on elastin biosynthesis were obtained when the vitamin was added as two to three divided doses every day. Recently, investigators have reported a novel form of ascorbate, ascorbate 2-phosphate, that appears to have a much longer biological half-life (16, 17). Preliminary findings confirm that using the more stable form of ascorbate, long exposure times and multiple doses are not necessary to obtain significant reduction of TE production.²

Bergethon et al. (25) showed that ascorbate and isoascorbate had equivalent effects on elastin accumulation and elastin prolyl hydroxylation, while another reducing agent, dithiothreitol, had no effect. The effect of ascorbate appeared to be specific to the active form of the molecule. Our present findings show that another physiologic reducing agent, glutathione, did not have an effect on elastin production except at toxic levels, and dehydroascorbate, which also has reducing potential but lacks prolyl hydroxylase cofactor activity, did not affect elastin production in cultured cells.

The effect of ascorbate on elastin production did depend on the state of cell differentiation. Inhibition was less evident in cells at low density and in a phase of rapid growth, a phase at which relatively low levels of elastin production are observed. As cells neared confluence (postconfluence in the case of smooth muscle cells), higher levels of elastin production developed, and ascorbate inhibition reached its maximal extent. Likewise, the effect of ascorbate on the more elastogenic thoracic aortic smooth muscle cells was much greater than in abdominal aortic smooth muscle cells. Although ascorbate had small effects on cell proliferation, as also reported by others (61), these changes were not sufficient to account for changes in elastin expression, and all data were normalized to cell number.

There did not appear to be any heterogeneity to the ascorbate response, at least in fibroblast populations that were examined by in situ hybridization. Changes in mRNA levels appeared to be uniform throughout the culture population. In vivo, the effects of excess ascorbate appear to be most prominent in the vessel wall (62), which may reflect the high state of elastogenesis. Similarly, supravalvular aortic stenosis appears to arise from the rate-limiting production of elastin in the aortic root due to a large truncation of one elastin allele (63).

Many previous studies on the effect of ascorbate on elastin did not discriminate between an effect upon synthesis or accumulation. One exception is the recent report that short term (48 h), low dose ascorbate (10 μg/ml) appears to increase the stability of secreted tropoelastin without an effect on mRNA levels (64). Overhydroxylation of tropoelastin may affect its turnover or incorporation into the extracellular matrix. The present studies show that most of the long term effects of ascorbate can be ascribed to biosynthesis. Given this observation, we wished to know whether the regulation was a pre- or posttranslational event. Studies of the secretory pathway of elastin had suggested that impaired secretion might lead to a form of negative feedback regulation at the transcript level (65). It has also been proposed (25) that the overhydroxylation of elastin, which might occur in ascorbate-supplemented conditions, could alter the rate of secretion as a result of conformational alterations that affect secretion and that ultimately feed back to down-regulate mRNA levels. In addition, there is experimental evidence that the presence of hydroxyproline in typical elastin primary sequences alters its thermodynamic behavior (66). Investigations of the effect of ascorbate on collagen I transcript levels have shown regulation at the level of both mRNA stability and transcription (12). Although the majority of studies, using acute ascorbate treatment, show substantial transcriptional activation (15, 17, 67), our own data emphasize the role of mRNA stability. The prolonged exposure to ascorbate may attenuate the transcriptional response. The mechanisms for altering collagen transcript stability are unknown (12), and a number of pathways have been proposed to explain the effect of ascorbate on transcriptional activity (68). A recent hypothesis has invoked the ability of ascorbate to generate lipoperoxides, which produce reactive aldehydes that could act upon the transcriptional machinery (67, 69–71). For example, malondialdehyde has been shown in one study to modulate the production of collagen in fibroblasts (67). This area of investigation is controversial, since a very recent report has shown that cell-impermeant agents such as desferroxamine and EDTA can block the production of lipoperoxides without affecting the ability of ascorbate to stimulate collagen production (72). It is not known whether lipoperoxidation mediates the effects of ascorbate on elastin production.

Elastin mRNA abundance is regulated by both transcription and mRNA stability (55, 73). Transcriptional regulators include insulin-like growth factor-1 (+), tumor necrosis factor-α (−), transforming growth factor-β (+), retinoic acid (+), and

² O. Zoia and J. M. Davidson, unpublished observation.
interleukin-1 (−). mRNA stability is strongly modulated by agents that affect protein kinase C (phorbol esters), transforming growth factor-β, and 1,25-dihydroxyvitamin D₃ (55). Since mRNA stability is a major factor in elastin regulation, it was reasonable to ask whether the vitamin had an effect on elastin mRNA stability. As the biosynthetic responses of collagen I and elastin are opposite, we could readily determine whether their stabilities were also inversely affected by ascorbate treatment. Under conditions of ascorbate withdrawal, COL1A2 mRNA was degraded more rapidly than mRNAE, while under conditions of ascorbate supplementation, COL1A2 mRNA was stabilized markedly (t₁₀ increased 4.9-fold, from 7 to 33 h) as mRNAE stability declined by a factor of 2. Since the concentration of mRNAE fell more rapidly in the presence of continuous reaction conditions for the control of accumulation of these two proteins during inflammatory events and subsequent tissue repair.

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J. Biol. Chem. 1997, 272:345-352.
doi: 10.1074/jbc.272.1.345

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