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Underhydroxylated minor cartilage collagen precursors cannot form stable triple helices

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Matrix-free cells from chick-embryo sterna were incubated with various concentrations of 2,2′-bipyridyl, an iron chelator that inhibits prolyl hydroxylase and lysyl hydroxylase. At concentrations in the region of 0.1 mM, significant effects on cartilage collagen hydroxylation and secretion were observed. When the underhydroxylated collagens were subsequently digested with chymotrypsin or chymotrypsin plus trypsin at 4 °C for 15 min, the minor cartilage collagen precursors (namely types IX and XI) were extensively degraded; type II procollagen was only partially susceptible and was converted into underhydroxylated collagen. The results demonstrate that there were significant differences in triple-helix stability among cartilage collagens such that the underhydroxylated minor collagen precursors were unable to attain a native structure under conditions where type II procollagen was successful.

INTRODUCTION

Gustavson (1955) originally postulated that 4-hydroxyproline plays an important role in stabilizing the triple-helical structure of collagen (and procollagen). Since then, it has been shown that underhydroxylated type I procollagen, for example, thermally denatures at a temperature approx. 15 °C lower than its hydroxylated counterpart (Jimenez *et al.*, 1973; Berg & Prockop, 1973; Rosenbloom *et al.*, 1973). It is proposed, but not proven, that 4-hydroxyproline gives added stability to the collagen helix by permitting the formation of intramolecular hydrogen bonds (Ramachandran *et al.*, 1973). Despite the lack of 4-hydroxyproline, underhydroxylated type I pro-α-chains can still assemble intracellularly into disulphide-linked trimers (Jimenez *et al.*, 1974; Fessler & Fessler, 1974), and a portion of these will become helical, as measured by proteinase-resistance, if the temperature is lowered below the denaturation temperature (Fiedler-Nagy *et al.*, 1981). It has further been reported that underhydroxylated type II (Uitto & Prockop, 1974; Harwood *et al.*, 1977; Oohira *et al.*, 1979) pro-α-chains can similarly assemble intracellularly into disulphide-bonded non-helical trimers at 37 °C, but there is no information on the ability of these chains to become helical upon lowering the temperature.

To determine whether cartilage collagen precursors behaved similarly to type I procollagen, experiments were conducted with 2,2′-bipyridyl in short-term suspension cultures of 17-day chick-embryo sternal cells. These cells have been shown to synthesize type II procollagen as well as precursors to cartilage collagen types IX and XI (1α, 2α and 3α), but not type X (Clark & Richards, 1985). In this context, the term 'precursor' refers to the initially synthesized collagenous molecule as distinguished from the molecule generated by the action of proteinase *in vitro*. It does not presume that the molecule is ever physiologically converted into another species. For example, type IX collagen may not be further processed after secretion (van der Rest *et al.*, 1985). The data provided in the present paper show that 2,2′-bipyridyl affected the hydroxylation and secretion of all cartilage collagen precursors, but differentially affected their ability to form a stable triple helix.

EXPERIMENTAL

Materials

Trypsin (type IX) and 2,2′-bipyridyl were purchased from Sigma Chemical Co. Unless otherwise indicated, all other materials are the same as those described previously (Clark & Richards, 1985).

Collagen precursor preparation

Cells isolated from 17-day chick-embryo sterna were incubated at 37 °C as described previously (Dehm & Prockop, 1973; Clark & Richards, 1985) in the absence or in the presence of various concentrations of 2,2′-bipyridyl. The suspension cultures contained approx. 10^7 cells/ml of Krebs II medium supplemented with 10% (v/v) dialysed fetal-calf serum, 1 mM-Na_2EDTA, 5 μl of aprotinin/ml, 50 μg of β-aminopropionitrile/ml and 50 μg of sodium ascorbate/ml. After a 30 min pre-incubation, the cells were metabolically labelled with [^{14}C]proline (4 μCi/ml) for 2 h. Cycloheximide (final concentration 0.8 mM) and proteinase inhibitors were added at this time, and incubation was continued for 1 h to allow any partially completed molecules to be secreted. The final concentrations of proteinase inhibitors were 10 mM-Na_2EDTA, 10 mM-*N*-ethylmaleimide, 1 mM-phenylmethanesulphonyl fluoride and 1 mM-benzamidine hydrochloride. The incubation mixture was immediately centrifuged at 900 *g* to separate cells and medium. The medium was dialysed at 4 °C against 0.4 M-NaCl/0.1 M-Tris/HCl buffer, pH 7.4; the cells were resuspended in 1 ml of 0.5 M-acetic acid containing 20 μg of pepstatin and frozen for at least 24 h. Cells were subsequently thawed, briefly sonicated and dialysed sequentially at 4 °C against 0.5 M-acetic acid, deionized

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water and 0.4 M-NaCl/0.1 M-Tris/HCl buffer, pH 7.4. Samples of the cell homogenate and medium were taken for counting of total ^{14}C radioactivity incorporation, and portions were assayed for 4-hydroxy[^{14}C]proline content (Juva & Prockop, 1966). In some experiments the collagen precursors were further purified by precipitation at 40 % saturation of $(NH_4)_2SO_4$ (Clark & Richards, 1985).

Collagen assay

The amount of [^{14}C]proline-labelled collagen in medium and cell samples was determined by using digestion with bacterial collagenase (Peterkofsky *et al.*, 1982).

Gel electrophoresis and fluorography

Labelled proteins were analysed by SDS/polyacrylamide-gel electrophoresis in 6.5 % slab gels under reducing and non-reducing conditions as described previously (Clark & Richards, 1985). To confirm the identity of underhydroxylated type II collagen, CNBr digestion and analysis of the resultant peptides by two-dimensional SDS/polyacrylamide-gel electrophoresis was performed essentially as described by Barsh *et al.* (1981). After electrophoresis the gels were processed for fluorography with En^{3}Hance (New England Nuclear), dried under vacuum and heat and exposed to RP Royal X-Omat film (Eastman Kodak) at −70 °C in a Cronex cassette (Dupont) for 24–48 h. In some instances, fluorograms were subjected to analysis with the use of a recording densitometer (Ortec).

Proteinase digestions

For chymotrypsin digestion, samples were incubated in 0.4 M-NaCl/0.1 M-Tris/HCl buffer, pH 7.4, containing 300 μg of tosyl-lysylchloromethane ('TLCK')-treated α-chymotrypsin/ml for 6 h at 10 °C. Digestion was stopped by the addition of tosylphenylalanylchloromethane ('TPCK') in methanol to a final concentration of 100 μg/ml and incubation was continued overnight at 4 °C. Digested material was removed by trichloroacetic acid precipitation, and the resultant precipitates were analysed by SDS/polyacrylamide-gel electrophoresis as described previously (Clark & Richards, 1985). Control samples were incubated without enzyme.

For chymotrypsin/trypsin digestion, the procedure of Fiedler-Nagy *et al.* (1981) was followed. Digestions were performed at 4 °C for 15 min, and the digests were directly analysed by SDS/polyacrylamide-gel electrophoresis. Control samples were incubated without enzyme.

RESULTS

Dose–response effect of 2,2′-bipyridyl on cartilage collagen hydroxylation and secretion

It can be seen that increasing concentrations of 2,2′-bipyridyl inhibited proline hydroxylation and cartilage collagen secretion in a dose-dependent manner (Fig. 1). The concentration of 2,2′-bipyridyl that resulted in a 50 % inhibition of both proline hydroxylation and collagen secretion was in the region of 0.1 mM. However, a detectable inhibition of the former could be observed at 25 μM, whereas the latter was first observed at 0.1 mM (Fig. 1). Electrophoretic analysis of the relatively small fraction of newly synthesized collagen that appeared in the medium in the presence of 2,2′-bipyridyl (cf. Table 1) showed that secretion of all the cartilage collagens (namely types II, IX and XI) was affected (results not shown). Interestingly, the secreted collagens did not show the differences in electrophoretic mobility associated with underhydroxylation (cf. Fig. 2), suggesting that their hydroxylation and secretion were apparently unaffected by 2,2′-bipyridyl. The reason for this is not known.

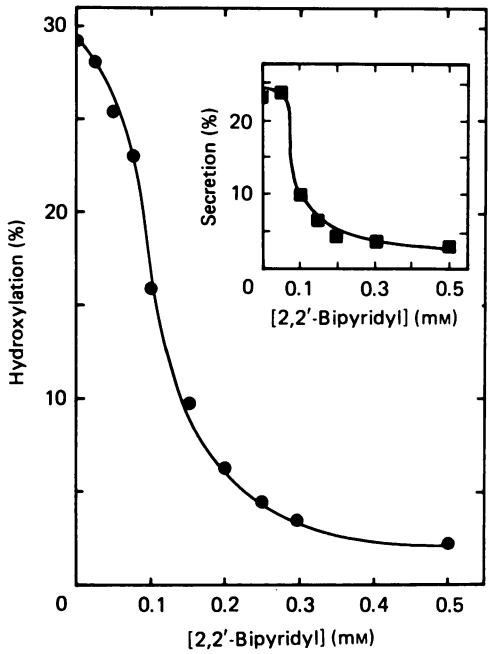

Fig. 1. Effects of 2,2′-bipyridyl concentration on the hydroxylation and secretion of cartilage collagens

Cartilage cells were incubated in the absence or in the presence of concentrations of 2,2′-bipyridyl ranging from 0.025 to 0.5 mM, and the medium and cells were isolated as described in the Experimental section. Samples of each were taken for analysis of 4-hydroxy[^{14}C]proline and for bacterial-collagenase-susceptible radioactivity. The results of the hydroxyproline assay of cells plus medium were expressed as percentage hydroxylation (100 × 4-hydroxy-[^{14}C]proline c.p.m./total ^{14}C c.p.m.) and are plotted as a function of 2,2′-bipyridyl concentration. Inset: the results of the collagen assay were expressed as percentage secretion (100 × collagen c.p.m. in medium/total collagen c.p.m.) and are plotted as a function of 2,2′-bipyridyl concentration.

Dose–response effect of 2,2′-bipyridyl on the electrophoretic mobility and proteinase-sensitivity of cartilage collagens

To assess the proteinase-sensitivity of the collagens synthesized in the presence of various concentrations of 2,2′-bipyridyl, portions of cell extracts from the above samples were digested with chymotrypsin at 10 °C and the products were analysed by SDS/polyacrylamide-gel electrophoresis as described in the Experimental section. At concentrations of 2,2′-bipyridyl up to 50 μM, there was no significant change in either the electrophoretic mobility or the proteinase-sensitivity of the various newly synthesized collagens (results not shown). However, at 0.1 mM-2,2′-bipyridyl there was a marked increase

in the mobility of all underhydroxylated collagens, which was most noticeable for pro-α1(II) (Fig. 2, compare lanes 1 and 2); the mobility of this component was further increased at 0.2 mM-2,2′-bipyridyl (Fig. 2, compare lanes 2 and 3), but did not increase at higher concentrations of the inhibitor (Fig. 2, compare lanes 3 and 4).

In addition to the above changes in mobility caused by the decrease in hydroxylation, the most marked effect of underhydroxylation on the minor cartilage collagens was on their proteinase-sensitivity. In the absence of 2,2′-bipyridyl (Fig. 2, lane 1) the presence of the digestion products of types IX (61 kDa) and XI (1α, 2α and 3α) collagen are readily identified (Clark & Richards, 1985). As the concentration of 2,2′-bipyridyl increased, so did the degradation of these components, such that at 0.2 mM the presence of the minor cartilage collagens was barely detectable (Fig. 2, compare lanes 2 and 3); at 0.3 mM (lane 4), only underhydroxylated α1(II) was present. The identity of the component in lane 4 was shown to be underhydroxylated α1(II) by analysis of CNBr-cleavage peptides (results not shown). These results suggest that the underhydroxylated minor cartilage collagens were proteinase-sensitive under conditions where type II procollagen was not.

The remaining experiments were performed with 0.5 mM-2,2′-bipyridyl, the concentration at which the maximal inhibition of proline hydroxylation and collagen secretion was observed (Fig. 1).

Quantitative effect of 0.5 mM-2,2′-bipyridyl on total protein synthesis and on cartilage collagen synthesis, hydroxylation and secretion

In a typical experiment, continuous labelling of chondroblast cultures in the absence or in the presence of 0.5 mM-2,2′-bipyridyl showed that total incorporation of ^{14}C into protein was only slightly decreased (91% of control) and incorporation into collagen was only moderately decreased (78% of control) in the presence of the inhibitor (Table 1). In contrast, there was a marked effect on proline hydroxylation (approx. 3% of control) and collagen secretion (21% of control) consistent with reported effects of 2,2′-bipyridyl on chick tibial cartilage cultures (Bhatnagar & Prockop, 1966). The difference between some of the values in Fig. 1 and Table 1 is representative of the variations between different cell preparations and experiments. It should be noted, however, that the hydroxylation values shown in Table 1 were obtained from purified samples, in contrast with those in Fig. 1.

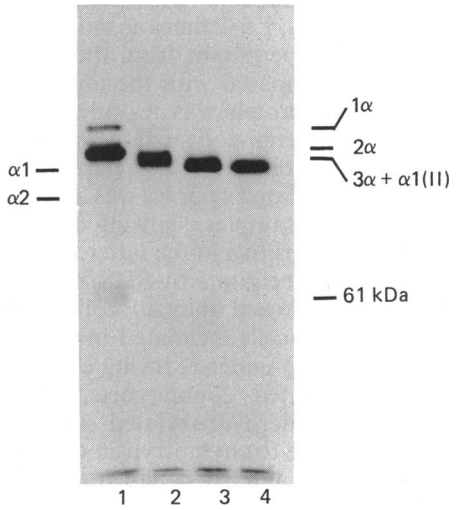

Fig. 2. Fluorescence autoradiographic analysis of the effects of 2,2′-bipyridyl concentration on the electrophoretic mobility and chymotrypsin-sensitivity of cartilage collagens

Cartilage cells were incubated in the absence or in the presence of various concentrations of 2,2′-bipyridyl, and the cell-associated collagens were extracted, purified by $(NH_4)_2SO_4$ precipitation and digested with chymotrypsin as described in the Experimental section. Digests containing equal amounts of radioactivity (2000–4000 c.p.m.) were analysed by electrophoresis on a 6.5% polyacrylamide slab gel under reducing conditions. The migration positions of chains from type II [α1(II)], type IX (61 kDa) and type XI (1α, 2α and 3α) collagens are shown on the right; the migration positions of pepsin-digested type I collagen chains (α1 and α2) are shown on the left. The concentration of 2,2′-bipyridyl used in each incubation is: lane 1, 0 mM; lane 2, 0.1 mM; lane 3, 0.2 mM; lane 4, 0.3 mM.

Table 1. Distribution of total [^{14}C]proline-labelled protein and collagen synthesized in the presence or in the absence of 2,2′-bipyridyl

Freshly isolated chondroblasts were isolated from 100 sterna dissected from 17-day chick embryos as described in the Experimental section. The cells were counted with a haemocytometer (450×10^8 cells) and divided equally into two flasks. One flask was made 0.5 mM in 2,2′-bipyridyl, and both flasks were treated as described in the Experimental section. Samples were analysed for total ^{14}C, ^{14}C-labelled collagen (Peterkofsky *et al.*, 1982) and 4-hydroxy[^{14}C]proline (Juva & Prockop, 1966). The analyses of hydroxy[^{14}C]proline/total ^{14}C ratios were performed on proteins precipitated from medium or cell extracts by 40%-saturated $(NH_4)_2SO_4$. Values in parentheses represent the percentages of the total radioactivity (c.p.m.) in the medium of each sample.

Treatment	Fraction	$10^{-5} \times$ Total ^{14}C-labelled protein (c.p.m.)	$10^{-5} \times$ Total ^{14}C-labelled collagen (c.p.m.)	100 × Hydroxy-[^{14}C]proline/ total ^{14}C (%)
2,2′-Bipyridyl absent	Cell	3.69	1.91	34.3
	Medium	1.14 (24%)	0.72 (27%)	39.6
2,2′-Bipyridyl present	Cell	3.92	1.91	1.4
	Medium	0.46 (10%)	0.15 (7%)	0.7

Further studies on the proteinase-sensitivity of underhydroxylated cartilage collagen precursors

To document further the effect of proteinase digestion on the minor cartilage collagen precursors, samples from homogenates of cells that had been incubated in either the absence or the presence of 0.5 mM-2,2′-bipyridyl were digested with chymotrypsin. After trichloroacetic acid precipitation of digests, 1.5–2 times as much radioactivity was present in the supernatant from the 2,2′-bipyridyl-treated sample as compared with the untreated sample, suggesting that more protein was degraded in the former. At the same time, bacterial-collagenase-susceptible radioactivity in the trichloroacetic acid precipitate of chymotrypsin digests of untreated samples was 2–3 times that in 2,2′-bipyridyl-treated samples, showing that more collagen was proteinase-sensitive in the latter. Analysis of the trichloroacetic acid precipitate by SDS/polyacrylamide-gel electrophoresis showed that all collagen precursor chains obtained from cells incubated in the presence of 2,2′-bipyridyl migrated under reducing conditions faster than their hydroxylated counterparts, and that the majority of the underhydroxylated minor collagen chains were susceptible to chymotrypsin digestion whereas underhydroxylated type II collagen chains were not (Clark & Richards, 1987).

It could be argued that, even at low temperatures, the relatively prolonged chymotrypsin digestion period (6 h) led to instabilities in the helical conformation of the minor cartilage collagens and to their subsequent digestion. To overcome this argument, short-term digestions were performed with a mixture of chymotrypsin and trypsin, a combination found to be an efficacious proteolytic probe for triple-helical conformation (Bruckner & Prockop, 1981). The effect of this digestion protocol on the underhydroxylated sample (Fig. 3) was similar to that observed previously for long-term digestions with chymotrypsin (Clark & Richards, 1987). Again it appeared as if the underhydroxylated minor cartilage collagens were nearly completely degraded whereas underhydroxylated type II procollagen was partially resistant and was converted into underhydroxylated collagen (compare lane 4 with lane 3 in Fig. 3). An unexpected observation was that hydroxylated type IX precursor chains were relatively resistant to digestion under conditions where 1α, 2α, 3α and α1(II) chains were generated from their respective precursors (compare lane 2 with lane 1 in Fig. 3). Specifically, only a small amount of 61 kDa material was observed, in contrast with the long-term digestions with chymotrypsin, where the 97 kDa and 78 kDa components were completely converted into the 61 kDa component(s) (Clark & Richards, 1987). Bruckner *et al.* (1985) similarly noted that neither helical nor non-helical regions of type IX collagen are digested with trypsin at 25 °C.

Effect of 2,2′-bipyridyl on disulphide-bond formation

The failure to form interchain disulphide bonds could indirectly compromise the ability of underhydroxylated chains to form a triple helix upon cooling. Therefore a variety of untreated and 2,2′-bipyridyl-treated samples were analysed by SDS/polyacrylamide-gel electrophoresis under non-reducing conditions. The results showed that the vast majority of the collagenous components (74% of the untreated and 81% of the 2,2′-bipyridyl-treated sample) retained their disulphide bonds and migrated as aggregates near the top of the gel (results not shown; Clark & Richards, 1987).

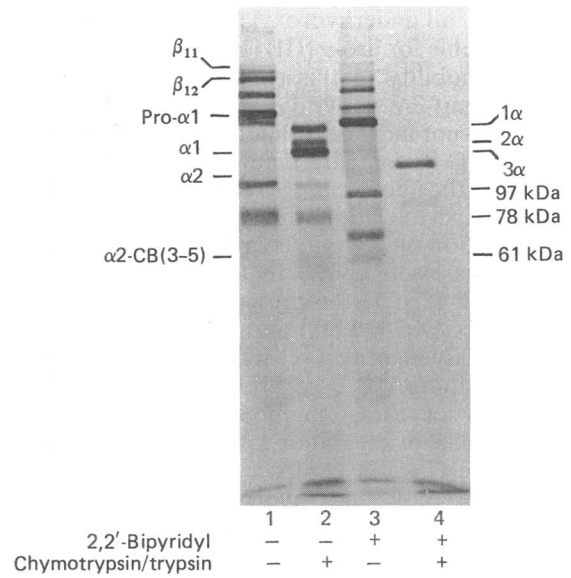

Fig. 3. Fluorescence autoradiographic analysis of the chymotrypsin-plus-trypsin-susceptibility of collagen precursors synthesized in the presence or in the absence of 0.5 mM-2,2′-bipyridyl

The preparation of samples is described in the legend to Fig. 2. Samples were digested with 100 μg of chymotrypsin/ml plus 10 μg of trypsin/ml; control samples contained no enzyme. Digestion was stopped as described by Fiedler-Nagy *et al.* (1981), and digests were immediately analysed by electrophoresis on a 6.5% polyacrylamide slab gel under reducing conditions. Each lane contained 9700 c.p.m. The migration positions of type I collagen components are shown on the left and of minor collagen components on the right. The presence (+) or absence (−) of 2,2′-bipyridyl in the original culture and of enzyme in the sample analysed is shown at the bottom.

DISCUSSION

Experiments with interstitial collagens (primarily type I) have led to the generally accepted conclusions that 4-hydroxyproline plays a critical role in stabilizing the triple helix under physiological conditions, and that non-helical collagen is inefficiently secreted as a non-functional protein that cannot be used for fibril formation (for review see Prockop *et al.*, 1976). In order to investigate the role of 4-hydroxyproline in the molecular assembly and secretion of cartilage collagens, chondrocytes from 17-day chick-embryo sterna were incubated with 2,2′-bipyridyl to inhibit the hydroxylation of proline and lysine residues. Since these cells synthesize and secrete types II, IX and XI collagen (Clark & Richards, 1985, 1987), it was possible to compare the effects of underhydroxylation on collagens of distinctly different structures (for review see Mayne & Irwin, 1986).

Our results show that incubation in the presence of 2,2′-bipyridyl affected all cartilage collagen precursors in a dose-dependent manner. For example, proline hydroxylation was significantly affected at 25 μM-2,2′-bipyridyl, a value similar to that found for type I

procollagen (Dehm & Prockop, 1971; Rosenbloom *et al.*, 1973). Changes in electrophoretic mobility and proteinase-sensitivity, however, were not observed at less than 0.1 mM-2,2′-bipyridyl, a concentration that results in approximately 50% inhibition of proline hydroxylation and collagen secretion. These findings suggest that a low degree of underhydroxylation does not impair helix formation.

At concentrations of 2,2′-bipyridyl greater than 50 μM, all cartilage collagen chains showed a significantly faster electrophoretic mobility than their hydroxylated counterparts. This indicates that all of these chains were underhydroxylated. This difference in electrophoretic mobility has been attributed to the lower molecular masses of the unhydroxylated chains due to the absence of hydroxy groups on both proline and lysine residues, and of galactosyl and glucosylgalactosyl moieties attached to hydroxylysine residues (Fessler & Fessler, 1974; Harwood *et al.*, 1977; Kao *et al.*, 1979). The significant alteration in mobility seen in the type IX chains (97 and 78 kDa) thus could indicate that they contained relatively large numbers of glycosylated hydroxylysine similar to α1(II) chains. This would be consistent with the high content of hydroxylysine in this collagen (Reese & Mayne, 1981).

The most significant observation in these studies, however, was the fact that underhydroxylated minor cartilage collagen precursors were susceptible to proteinase digestion under conditions where underhydroxylated type II procollagen was not. A similar observation has been made for type X collagen (Capasso *et al.*, 1984). This suggests that the molecular properties of these underhydroxylated minor cartilage collagen precursors were distinctly different from those of the interstitial procollagens. In type I procollagen, for example, the newly synthesized underhydroxylated chains retain their ability to form disulphide-bonded trimers through their *C*-terminal propeptides and, when lowered below their denaturation temperature, a portion of these trimers can become proteinase-resistant (i.e. triple-helical) (Prockop *et al.*, 1976, and references cited therein; Fiedler-Nagy *et al.*, 1981). Our results show that underhydroxylated type II procollagen behaved in a similar fashion.

It has been suggested for interstitial procollagens that chain association and subsequent disulphide-bonding are the limiting steps in helix formation (Uitto & Prockop, 1973, 1974; Gerard *et al.*, 1981). Nevertheless, the present finding that the majority of underhydroxylated minor cartilage collagen precursors were disulphide-bonded, yet proteinase-sensitive, would suggest that the role of these linkages, although necessary, is not sufficient for stable helix formation in other collagen types. The observed proteinase-sensitivity of the minor cartilage collagens correlated with the inhibition of proline hydroxylation and collagen secretion. It must be emphasized that, since proteinase-sensitivity is a very stringent test for collagen helicity, digestion of collagen does not imply that no triple helix formed; one can only surmise that, if a helical conformation were attained, it must not have been fully aligned. Thus it must be concluded that the minor collagen precursors did not form stable triple helices (Bruckner & Prockop, 1981) under conditions where type II procollagen did. However, one can only speculate at this time about the reason for this behaviour.

The structure of type IX collagen appears to be distinctly different from the other cartilage collagen types in that the native molecule contains three collagenous domains, each flanked by non-collagenous domains. Interchain disulphide bonds are present in the *C*-terminal non-collagenous domain (NC 1) and also in NC 3 (van der Rest *et al.*, 1985). If it is assumed that chain association is initiated at the *C*-terminus (there is no evidence either to confirm or to refute this), then the disulphide bonds in NC 1 may be important in stabilizing the newly formed trimer. Since our results show that a significant portion of the underhydroxylated type IX chains were associated and disulphide-bonded, the failure to form a stable triple helix at low temperature could be due to an inherent instability of the collagenous domains, which would be exacerbated by the presence of relatively flexible non-collagenous domains.

Less is known about the structure of the precursor(s) to type XI collagen. Clark & Richards (1985) report that the molecule contains interchain disulphide bonds in a proteinase-sensitive region (or regions), but the precise location is not known. Previous results have further shown that the proteinase-resistant chains are similar in size to interstitial collagen chains (Burgeson *et al.*, 1982). This argues against the presence of internal non-collagenous domains as found in type IX collagen. Since the present results also show that a significant portion of these chains were associated and disulphide-bonded, the failure to form a stable triple helix at low temperature could be due to an inherent instability of the constituent underhydroxylated pro-α-chains. The reason for such an instability probably will not be discovered until more information about the structure and chain composition of type XI collagen is obtained. Inasmuch as the 1α- and 2α-chains are reported to be similar to α1(V) and α2(V) respectively (Burgeson *et al.*, 1982), it would be interesting to know the effect of proteinase digestion on underhydroxylated type V procollagen.

In conclusion, the underhydroxylated precursors to types IX and XI collagens were susceptible to extensive proteinase digestion under conditions where underhydroxylated type II procollagen was not. The reason for the failure of the underhydroxylated minor cartilage collagens to form a stable triple helix can be gleaned only after further structural studies on these molecules. Nonetheless, whatever the reason, the proteinase-susceptibility of these molecules suggests that there may be differences in the mechanism of their chain assembly and stabilization.

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REFERENCES

Barsh, G. S., Peterson, K. E. & Byers, P. H. (1981) Collagen Relat. Res. **1**, 543–548

Berg, R. A. & Prockop, D. J. (1973) Biochem. Biophys. Res. Commun. **52**, 115–120

Bhatnagar, R. S. & Prockop, D. J. (1966) Biochim. Biophys. Acta **130**, 383–392

Bruckner, P. & Prockop, D. J. (1981) Anal. Biochem. **110**, 222–226
Bruckner, P., Vaughan, L. & Winterhalter, K. H. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 2608–2612
Burgeson, R. E., Hebda, P. A., Morris, N. P. & Hollister, D. W. (1982) J. Biol. Chem. **257**, 7852–7856
Capasso, O., Quarto, N., Descalzi-Cancedda, F. & Cancedda, R. (1984) EMBO J. **3**, 823–827
Clark, C. C. & Richards, C. F. (1985) Collagen Relat. Res. **5**, 205–223
Clark, C. C. & Richards, C. F. (1987) in Development and Diseases of Cartilage and Bone Matrix (Sen, A. & Thornhill, T., eds.), pp. 87–96, Alan R. Liss, New York
Dehm, P. & Prockop, D. J. (1971) Biochim. Biophys. Acta **240**, 358–369
Dehm, P. & Prockop, D. J. (1973) Eur. J. Biochem. **35**, 159–166
Fessler, L. I. & Fessler, J. H. (1974) J. Biol. Chem. **249**, 7637–7646
Fiedler-Nagy, C., Bruckner, P., Hayashi, T. & Prockop, D. J. (1981) Arch. Biochem. Biophys. **212**, 668–677
Gerard, S., Puett, D. & Mitchell, W. M. (1981) Biochemistry **20**, 1857–1865
Gustavson, K. H. (1955) Nature (London) **175**, 70–74
Harwood, R., Merry, A. H., Woolley, D. S., Grant, M. E. & Jackson, D. S. (1977) Biochem. J. **161**, 405–418
Jimenez, S. A., Harsch, M., Murphy, L. & Rosenbloom, J. (1974) J. Biol. Chem. **249**, 4480–4486
Jimenez, S., Harsch, M. & Rosenbloom, J. (1973) Biochem. Biophys. Res. Commun. **52**, 106–114
Juva, K. & Prockop, D. J. (1966) Anal. Biochem. **15**, 77–83
Kao, W. W.-Y., Prockop, D. J. & Berg, R. A. (1979) J. Biol. Chem. **254**, 2234–2243
Mayne, R. & Irwin, M. H. (1986) in Articular Cartilage Biochemistry (Kuettner, K. E., Schleyerbach, R. & Hascall, V. C., eds.), pp. 23–38, Raven Press, New York
Oohira, A., Nogami, H., Kusakabe, A., Kimata, K. & Suzuki, S. (1979) J. Biol. Chem. **254**, 3576–3583
Peterkofsky, B., Chojkier, M. & Bateman, J. (1982) in Immunochemistry of the Extracellular Matrix (Furthmayr, H., ed.), vol. 2, pp. 19–47, CRC Press, Boca Raton
Prockop, D. J., Berg, R. A., Kivirikko, K. I. & Uitto, J. (1976) in Biochemistry of Collagen (Ramachandran, G. N. & Reddi, A. H., eds.), pp. 163–273, Academic Press, New York
Ramachandran, G. N., Bansal, M. & Bhatnagar, R. S. (1973) Biochim. Biophys. Acta **322**, 166–171
Reese, C. A. & Mayne, R. (1981) Biochemistry **20**, 5443–5448
Rosenbloom, J., Harsch, M. & Jimenez, S. (1973) Arch. Biochem. Biophys. **158**, 478–484
Uitto, J. & Prockop, D. J. (1973) Biochem. Biophys. Res. Commun. **55**, 904–911
Uitto, J. & Prockop, D. J. (1974) Biochemistry **13**, 4586–4591
van der Rest, M., Mayne, R., Ninomiya, Y., Seidah, N. G., Chretien, M. & Olsen, B. R. (1985) J. Biol. Chem. **260**, 220–225

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