Richards, D. A., Rodgers, J. R., Supowit, S. C., & Rosen, J. M. (1981a) J. Biol. Chem. 256, 526–532.
Richards, D. A., Blackburn, D. E., & Rosen, J. M. (1981b) J. Biol. Chem. 256, 533–538.
Richardson, R. H., & Brew, K. (1980) J. Biol. Chem. 255, 3377–3385.
Shechter, Y., Patchornick, A., & Burnstein, Y. (1974) J. Biol. Chem. 249, 413–419.

Isolation and Partial Characterization of the Amino-Terminal Propeptide of Type II Procollagen from Chick Embryos†

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ABSTRACT: The amino-terminal propeptide from type II procollagen was isolated from organ cultures of sternal cartilages from 17-day-old chick embryos. The procedure provided the first isolation of the propeptide in amounts adequate for chemical characterization. The propeptide had an apparent molecular weight of 18 000 as estimated by gel electrophoresis in sodium dodecyl sulfate. It contained a collagen-like domain as demonstrated by its amino acid composition, circular dichroism spectrum, and susceptibility to bacterial collagenase. One residue of hydroxylysine was present, the first time this amino acid has been detected in a propeptide. The peptide contained no methionine and only two residues of half-cystine. Antibodies were prepared to the propeptide and were used to establish its identity. The antibodies precipitated type II procollagen but did not precipitate type II procollagen from which the amino and carboxy propeptides were removed with pepsin. Also, they did not precipitate the carboxy propeptide of type II procollagen. The data demonstrated that the type II amino propeptide was similar to the amino propeptides of type I and type III procollagens in that it contained a collagen-like domain. It differed, however, in that it lacked a globular domain as large as the globular domain of 77–86 residues found at the amino-terminal ends of the proα1 chains of type I and type III procollagens.

Procollagens have been identified as precursors of three interstitial collagens, types I, II, and III. Several of the type-specific N and C propeptides[1] have been characterized. The primary structure has been determined for the N-terminal propeptide of the proα1(I) chain in calf (Hörlein et al., 1979), sheep (Rohde & Timpl, 1979), and chick embryos (Pesciotta et al., 1980) and for the N-terminal propeptide of the proα1(III) chain from calf [A. Brandt, D. Hörlein, P. Bruckner, R. Timpl, P. P. Fietzek, and R. W. Glanville, unpublished results; see Timpl & Glanville (1981)]. The N propeptides for both proα1(I) and proα1(III) chains have an N-terminal globular domain of 77–86 amino acid residues, followed by a collagen-like domain of about 40 amino acid residues [for review, see Timpl & Glanville (1981)]. The collagen-like domain is joined to the α chain by a short non-collagen sequence of 2–8 amino acids. The N propeptide of the proα2(I) chain has not been completely characterized, but in sheep (Becker et al., 1977) and chick embryos (Tuderman et al., 1978; Morris et al., 1979) it lacks the N-terminal globular domain and consists of about 60 amino acid residues that are collagen-like in structure. In rat, the N propeptide of the proα2(I) appears to be about the same size as the N propeptide of proα1(I) and proα1(III) and therefore may contain the globular domain (Smith et al., 1977). In the case of type II procollagen, the presence of an N-terminal propeptide has been established (Olsen et al., 1976; Merry et al., 1976; Uitto et al., 1977), and it has been shown to be about two-thirds the size of the N propeptides of proα1(I) or proα1(III) chains (Tuderman et al., 1978). Also, biosynthetic studies suggest that it contains mannose, which is not found in the N-terminal propeptides of either type I or type III procollagen (Guzman et al., 1978). We here report the first isolation of the N propeptide of type II procollagen in amounts adequate for chemical characterization.

Experimental Procedures

Materials

Dulbecco's modified Eagle's medium, Eagle's minimal essential medium, streptomycin, penicillin, trypsin, and Freund's complete and incomplete adjuvants were products of Gibco, Inc. The radioisotopes used were New England Nuclear's mixture of ^{14}C-labeled amino acids and New England Nuclear's [^{14}C]proline, 240 μCi/μmol. DEAE-cellulose (DE-52) and CM-cellulose (CM-52) were purchased from Whatman, Inc., and Bio-Gel P-2 (200–400 mesh) was purchased from Bio-Rad Laboratories. Advanced Biofactures Corp. provided the bacterial collagenase. Protein A–Sepharose 4B was purchased from Pharmacia Fine Chemicals, and the hemocyanin used was Calbiochem's A grade. Dr. Bjørn Olsen kindly provided the sheep anti-rabbit IgG (Olsen et al., 1977).

Methods

Purification of the Type II N Propeptide. Type II propeptides were isolated by a modification of the organ culture system developed by Olsen et al. (1977). Sterna were dissected from 40 dozen 17-day-old chick embryos and cleaned of adhering perichondrial tissue. The whole sterna were incubated in a Dulbecco's modified Eagle's medium supplemented with 40 μg/mL sodium ascorbate, 60 μg/mL β-aminopropionitrile, and 1–2 μCi/mL radioisotope. The incubations were carried

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[1] Abbreviations: N propeptide, amino-terminal propeptide of a procollagen; C propeptide, carboxy-terminal propeptide of a procollagen; CD, circular dichroism; $NaDodSO_4$, sodium dodecyl sulfate; DEAE, diethylaminoethyl; CM, carboxymethyl; IgG, immunoglobulin G; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane.

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out for 4 or 24 h in a 10% CO_2–90% air atmosphere at 37 °C with shaking. In experiments using 4-h incubations, the medium was supplemented with 5 mM $CaCl_2$. In experiments using 24-h incubations, no $CaCl_2$ was added but the medium was supplemented with streptomycin and penicillin to final concentrations of 100 units/mL and 100 μg/mL, respectively. In some experiments, the 4 h incubation medium was a Krebs' medium II containing 111.2 mM NaCl, 15.7 mM sodium phosphate (pH 7.4), 5.4 mM KCl, 4.0 mM $NaHCO_3$, 1.3 mM $MgCl_2$, 1.6 mM KH_2PO_4, and 12 mM glucose.

At the end of the incubation period, the medium was separated from the sterna by centrifugation and cooled to 4 °C. Protease inhibitors were added to give final concentrations of 25 mM EDTA, 10 mM *N*-ethylmaleimide, 1 mM *p*-aminobenzamidine, and 1 mM phenylmethanesulfonyl fluoride (Hoffmann et al., 1976). The medium was then dialyzed against a 0.02 M Tris-HCl buffer, pH 8.6 at 23 °C, containing deionized 2 M urea. The dialyzed medium was applied to a 2.5 × 20 cm DEAE-cellulose column and eluted at 4 °C with an 800-mL linear gradient of 0–0.35 M NaCl. Flow rates were 120–140 mL/h. Fractions of 5 mL were collected and assayed by liquid scintillation counting. The recovery of ^{14}C-labeled material was 80%.

Propeptide fractions eluted from the column were desalted on a 4 × 50 cm P-2 column equilibrated in 0.2 M NH_4HCO_3 and lyophilized. The recovery of ^{14}C-labeled material was 85%.

Purification of Type II Procollagen. Type II procollagen was prepared from chondroblasts incubated in suspension culture. Chick sternal chondroblasts were isolated by the method of Dehm & Prockop (1973). Procollagen was purified from the cells by a modification of the method of Uitto (1977). Washed chrondroblasts were incubated at concentrations of 10^7 cells/mL in Krebs' medium II supplemented with 40 μg/mL sodium ascorbate, 60 μg/mL β-aminopropionitrile, and 1–2 μCi/mL [^{14}C]proline or ^{14}C-labeled amino acid mixture at 37 °C for up to 2 h. In some experiments, 20% fetal calf serum was added to the suspension to minimize proteolytic degradation. The medium was separated from the cells at the end of the incubation and treated with protease inhibitors (see above), and ammonium sulfate was added to a final concentration of 176 mg/mL. After overnight precipitation at 4 °C, the procollagen was sedimented by centrifugation at 15000*g* for 30 min at 4 °C. The procollagen was extracted and dialyzed into a 0.05 M Tris-HCl buffer, pH 8.0 at 23 °C, containing 2 M urea and 10 mM EDTA. The dialyzed procollagen was applied to a 1.5 × 10 cm DEAE-cellulose column equilibrated with the Tris–urea–EDTA buffer. The sample was eluted by a 200-mL linear salt gradient of 0–0.2 M NaCl at 4 °C with a flow rate of 100 mL/h. The peak fractions were pooled and dialyzed into a 0.1 M Tris-HCl buffer, pH 7.4 at 4 °C, containing 0.4 M NaCl and 10 mM EDTA. The procollagen was precipitated with ammonium sulfate as above, extracted into 0.1 M Tris-HCl buffer, pH 7.4 at 4 °C, containing 0.4 M NaCl and 10 mM EDTA, aliquoted, and stored frozen. The recovery of ^{14}C-labeled material was 55%.

Gel Electrophoresis. Polyacrylamide gel electrophoresis was performed under standard procedures. Routinely, slabs of 2-mm thickness were used with polyacrylamide concentrations of 6% or 15%. For fluorography, gels were impregnated with 2,5-diphenyloxazole, dried, and exposed to RP Royal X-OMAT X-ray film (Kodak) at −70 °C (Bonner & Laskey, 1974; Laskey & Mills, 1975). For protein staining, gels were immersed in a solution of 20% trichloroacetic acid and 0.025% Coomassie Brilliant Blue R for 1 h at room temperature. Gels were destained in 7.5% acetic acid and 15% methanol.

Collagenase Digestion. Lyophilized propeptide samples were dissolved and dialyzed against 0.4 M NaCl in 0.1 M Tris-HCl buffer, pH 7.4 at 4 °C, to which *N*-ethylmaleimide and $CaCl_2$ were added to final concentrations of 2.5 and 5.0 mM, respectively. Bacterial collagenase (100 units/mL) (form III; Advanced Biofactures Corp.) was added, and the sample was incubated for 6 h at 37 °C. At the end of the incubation, 3 volumes of polyacrylamide gel sample buffer containing 5 mM EDTA were added to the sample, which was then boiled and applied to a 15 polyacrylamide gel.

Amino Acid Analysis. Type II N propeptide was prepared for amino acid analysis by dialysis against 0.1 N formic acid, lyophilization, and hydrolysis under N_2 in 6 N HCl for 16 h at 116 °C. The amino acid analyzer was a Beckman Model 121B. The propeptide residues were reduced and alkylated for determination of half-cystine residues as carboxymethylcysteine. The protein samples were reduced for 4 h at room temperature under N_2 with 50 mM dithiothreitol in 100 mM Tris-HCl buffer (pH 8.6) containing 6 M urea. They were alkylated by adding sodium iodoacetate to a final concentration of 100 mM and incubating at room temperature in the dark for 1.5 h. The samples were desalted on a P-2 polyacrylamide column (Bio-Rad) in 0.2 M ammonium bicarbonate and lyophilized. They were also subjected to performic acid oxidation for the determination of methionine as methionyl sulfoxide and sulfone and for the determination of cysteic acid.

CD Studies. The CD spectrum of the N propeptide was measured in a Model 61 Varian spectropolarimeter and in water-jacketed cells. The temperature was controlled by a water bath with an automatic programmer (Neslab Instrument, Inc; Model TP-2), and the temperature was recorded by a thermistor inserted into the cell. Cells of 2-mm path length were used, and the propeptide concentration was typically 20–30 μg/mL. To ensure that the propeptides did not precipitate during the measurements, we counted 10-μL aliquots from the top of the cuvette before and after each measurement. The aliquots were then assayed for radioactivity by liquid scintillation counting. There was no apparent loss of labeled propeptide during the circular dichroism measurements.

Pepsin and Cyanogen Bromide Digestion. For pepsin digestion, ^{14}C-labeled procollagen samples were dialyzed against 0.1 N acetic acid overnight at 4 °C. The pepsin solution was prepared by dissolving 1 mg of lyophilized pepsin (Boehringer-Mannheim) in 1 mL of acetic acid. This pepsin solution was added to 10 mL of buffer (see above) containing about 200 μg of procollagen, and the sample was incubated for 2 h at 18 °C. The reaction was stopped by the dropwise addition of 0.5 N NaOH and titration to pH 7.4. The sample was dialyzed overnight at 4 °C against a 0.1 M Tris-HCl buffer, pH 7.4 (4 °C), containing 0.4 M NaCl. Aliquots of the procollagen digest were boiled in a final concentration of 4% $NaDodSO_4$ for 3 min, dialyzed into slab sample buffer, and applied to 15% polyacrylamide slab gels for visualization.

Cyanogen bromide digestion was performed with a modification of the method of Epstein (1974). The lyophilized propeptide was dissolved in 70% formic acid and flushed with nitrogen for 15 min. A 1000-fold molar excess of recrystallized cyanogen bromide was added to the samples, which were flushed with nitrogen for another 15 min. The incubation was then continued at 37 °C for 4 h.

Immunological Techniques. Antibodies to the purified type II N propeptide were prepared by intradermal injections of the purified peptide into white New Zealand male rabbits. The

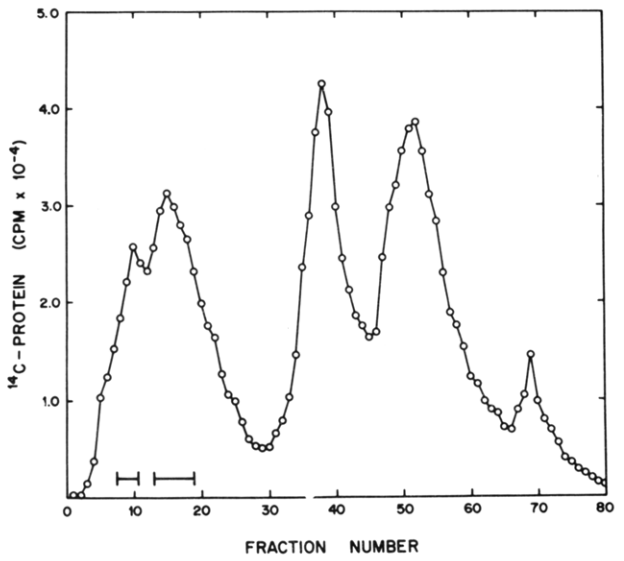

FIGURE 1: DEAE-cellulose chromatography of type II N propeptide from organ cultures of sternal cartilages. Conditions are as described in the text. Bar lines represent the leading edge and later fractions of the breakthrough volume containing the type II N propeptide. The salt gradient began in fraction 25.

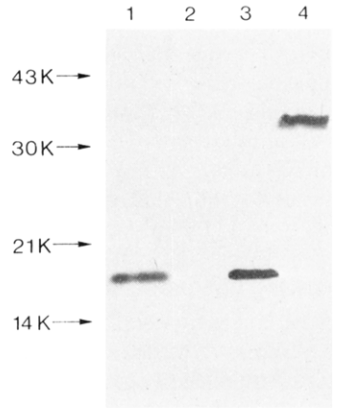

FIGURE 2: Fluorogram of polyacrylamide gel electrophoresis of type II N propeptide. Protein staining of the gel yielded similar results. Polyacrylamide concentration was 15%. (Lane 1) Isolated N propeptide; (lane 2) bacterial collagenase digest of N propeptide; (lane 3) cyanogen bromide digest of N propeptide; (lane 4) bacterial collagenase digest of type II procollagen.

propeptide was dissolved in phosphate-buffered saline, pH 7.4, and emulsified in Freund's complete adjuvant. Booster injections in incomplete adjuvant were given at 14–17-day intervals.

In some experiments, the type II N propeptide was covalently cross-linked with glutaraldehyde to hemocyanin to increase the immune response. The complex was prepared by a modification of the method of Avrameas & Ternyck (1969) and was dialyzed against phosphate-buffered saline before injection.

Direct-binding radioimmune assays were used to titer the immune sera developed in the rabbits. The immune complexes were precipitated with either protein A–Sepharose 4B (Pharmacia) or sheep anti-rabbit IgG (Nist et al., 1975; Olsen et al., 1977). Immune sera were incubated with their respective antigens for 1 h at room temperature. The protein A–Sepharose or the second antibody was added and the incubation continued at 4 °C for 24 h. The immune complexes were washed, extracted into 0.2 N HCl, and counted with Aquasol (New England Nuclear) as a scintillation cocktail. Alternatively, the complexes were boiled in 200 μL of a buffer containing 4% $NaDodSO_4$, 0.125 M Tris-HCl (pH 6.8), 10% glycerol, 0.025% Bromophenol Blue, and 10 μL of 2-mercaptoethanol. The boiled samples were dialyzed against this buffer and applied to a 15% polyacrylamide slab gel for visualization.

Results

Isolation of N Propeptide. Sternal cartilages from 17-day-old chick embryos were previously shown to synthesize type II procollagen in vitro (Dehm & Prockop, 1973; Uitto, 1977; Linsenmayer et al., 1979). Therefore, an organ culture system of this tissue was employed here to obtain the type II N propeptide. The incubation medium was treated with protease inhibitors and then immediately applied to a DEAE-cellulose column. The protein which was subsequently identified as the N propeptide was recovered in the breakthrough fractions of a DEAE-cellulose column (Figure 1).

$NaDodSO_4$ gel electrophoresis showed that the N propeptide recovered in the leading edge of the breakthrough fractions was homogeneous by the criteria of protein staining and radioactive fluorography (Figure 2). The latter half of the same peak contained several proteins (not shown). The N propeptide in these fractions could be purified to homogeneity by further chromatography on CM-cellulose (Curran & Prockop, 1982). The salt gradient eluted three major peaks of radioactivity (Figure 1). The first peak primarily contained a disulfide-linked trimer, which was identified as the C propeptide of type II procollagen by its amino acid composition and by the demonstration that antibodies to the peptide precipitated type II procollagen but not pepsin-treated type II procollagen nor the type II N propeptide isolated here. The further purification and characterization of the C propeptide will be described elsewhere (Curran & Prockop, 1982). The second peak eluting in the gradient (Figure 1) contained the type II C propeptide together with several other unidentified proteins. The last peak (Figure 1) was a high molecular weight protein that was not identified.

Preliminary experiments demonstrated that the yield of N propeptide was greater when the tissue was incubated for 24 h instead of 4 h. There was, however, partial degradation of the protein obtained after a 24-h incubation. Therefore, the incubation time was limited to 4 h for most experiments. The yield of N propeptide in such experiments was about 1.5 μg/chick embryo sternum. The purified and lyophilized N propeptide was stable at −20 °C for 3–4 weeks; with longer storage there was degradation, probably because of the presence of trace amounts of proteinases.

The nonreduced N propeptide migrated as a polypeptide with an apparent molecular weight of 18 000 as compared to standards of known globular proteins (Figure 2). There was no apparent change in migration after reduction with 2-mercaptoethanol. Tuderman et al. (1978) also obtained an apparent molecular weight of 18 000 for the N propeptide obtained by cleavage of type II procollagen with a partially purified procollagen N-proteinase. Under the same electrophoretic conditions employed here, the N propeptide of the proα1(I) chain migrated with an apparent molecular weight of about 20 000 unreduced and 23 000 reduced. Similar values were reported by Pesciotta et al. (1980).

Collagen-like Domain of the N Propeptide. Amino acid analysis of the purified N propeptide indicated that it was largely collagenous (Table I). About one-quarter of the residues were glycine. The peptide contained three residues of 4-hydroxyproline and one residue of hydroxylysine. The presence of hydroxylysine marks the first time that this residue

Table I: Amino Acid Composition of Type II N Propeptide[a]

amino acid	proα1(II) N propeptide[a]	proα1(I) N propeptide[b]
4-Hyp	3	5
Asp	5	21
Thr	4	6
Ser	6	8
Glu	15	19
Pro	9	18
Gly	23	25
Ala	8	7
½-Cys	2	10
Val	6	8
Met	0	0
Ile	6	8
Leu	7	8
Tyr	1	3
Phe	2	3
Hyl	1	0
Lys	6	4
His	1	1
Arg	5	5

[a] Values expressed as residues per peptide assuming a minimum of one residue of tyrosine, hydroxylysine, or histidine. Values are the mean of ten analyses on seven different preparations. [b] Values for type I N propeptide isolated from chick embryo tendons (Pesciotta et al., 1980).

has been found in a procollagen propeptide [see Timpl & Glanville (1981)]. Compared to the N propeptide of proα1(I) chains from chick embryos (Pesciotta et al., 1980), the type II N propeptide contained considerably fewer aspartate and half-cysteine residues. Otherwise, the compositions were similar. The peptide did not contain methionine. This observation was confirmed by the fact that there was no change in electrophoretic mobility of the peptide after treatment with cyanogen bromide (lane 3 in Figure 2).

The collagen-like structure was confirmed by digestion of the N propeptide by bacterial collagenase. After digestion, no band of radioactive peptide was seen on $NaDodSO_4$–polyacrylamide gels (lane 2 in Figure 2). Also, no collagenase-resistant peptide was seen even when the gel was treated with 20% trichloroacetic acid prior to processing for fluorography.

Based on the assumption of a minimum of 1 residue of tyrosine, hydroxylysine, or histidine/mol of N propeptide, we determined that the peptide contained 110 residues and had a mass of 11 082 daltons (Table I).

CD Spectrum. The presence of a collagen-like structure in the N propeptide was further confirmed by examining the peptide by CD. The spectrum of the isolated peptide showed a negative peak at 198 nm similar to the negative peak in this region seen with procollagens and collagens (Figure 3). It also had a small positive peak at about 221 nm as is seen with procollagens and collagens (Brown et al., 1969, 1972; Brodsky-Doyle et al., 1976; Hayashi et al., 1979; Gerard et al., 1981). After heat denaturation at 60 °C for 2 h, the spectrum became less negative below 210 nm but became more negative above 210 nm. Both these changes are again similar to those seen with procollagens and collagens.

The specific mean residue ellipticity at 198 nm of the undenatured peptide was −19 000 deg cm^2 dmol^{-1}. This value is less than the value for collagen of −60 000 deg cm^2 dmol^{-1} [see Hayashi et al. (1979)]. It is also less than the value of −27 000 deg cm^2 dmol^{-1} for the bovine type III Col 1–3 fragment, which consists of the disulfide-linked trimer of the N propeptide of type III procollagen (Bruckner et al., 1978). The lower specific mean residue ellipticity of the type II N propeptide might reflect the presence of α helix in a globular

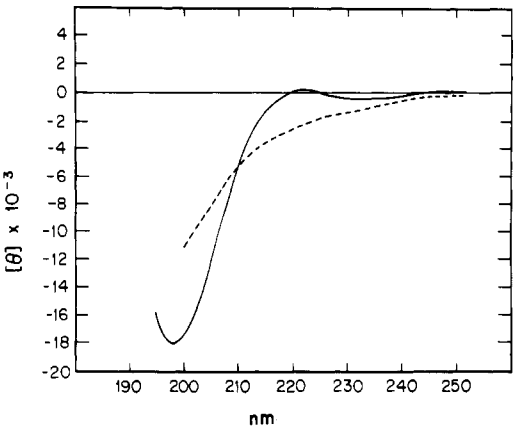

FIGURE 3: CD spectrum of type II N propeptide. Peptide was dissolved in 50 mM sodium phosphate buffer, pH 7.5, at a concentration of 25 μg/mL. Spectrum at 20 °C (—); spectrum after heat denaturation (---).

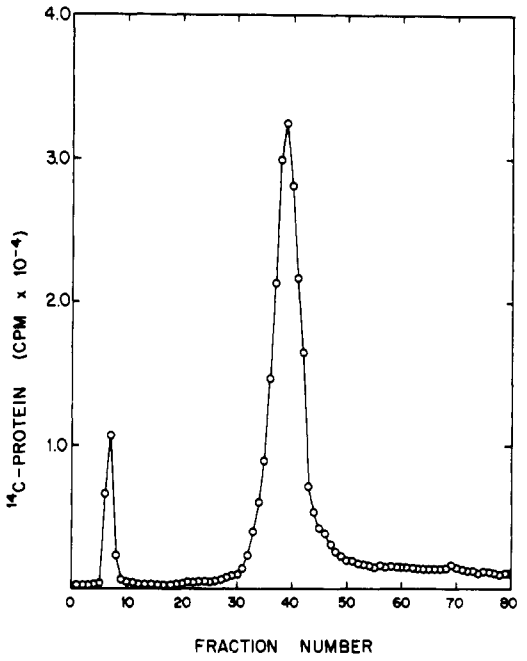

FIGURE 4: DEAE-cellulose chromatography of type II procollagen. Conditions are as described in the text. The salt gradient began in fraction 10.

domain. Under the conditions of the experiment here, however, the collagen domain of the N propeptide may not have been completely folded.

When the isolated N propeptide in a concentration of about 25 μg/mL was slowly heated, there was a relatively sharp transition over the range of 40–45 °C. However, the amounts of purified N propeptide were not sufficient to establish the transition temperature as a function of concentration [see Engel et al. (1977)].

Purification of Type II Procollagen. To identify the medium N propeptide, we compared it to the N propeptide of intact type II procollagen in terms of its susceptibility to bacterial collagenase and its immunological properties. Type II procollagen was prepared by incubating chick sternal chondroblasts in suspension culture and purifying the medium procollagen on a DEAE-cellulose column (Figure 4). Most of the ^{14}C-labeled material eluted as a sharp peak in fractions 33–44 of the salt gradient. As expected, gel electrophoresis in $NaDodSO_4$ after reduction of this peak gave a single band of mobility equal to that of the proα1 chain of type I (data not shown). The ^{14}C-labeled procollagen was digested with

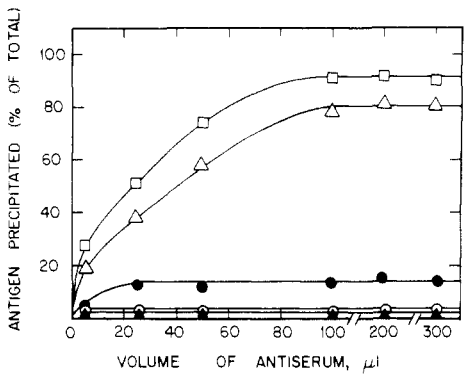

FIGURE 5: Direct-binding radioimmune assay using antiserum against type II N propeptide. About 4000 cpm of type II N propeptide (□), 5000 cpm of type II procollagen (△), 9000 cpm of type I procollagen (●), 6000 cpm of type II C propeptide (○), and 8000 cpm of pepsin-treated type II procollagen (▲) were used. Values are means from five or six experiments. The second antibody was 500 μL of sheep anti-rabbit IgG for most of the experiments, but 100 μL of protein A–Sepharose was used for samples of type II N and C propeptide in some experiments. Antisera used for procollagen precipitations were diluted 1:10.

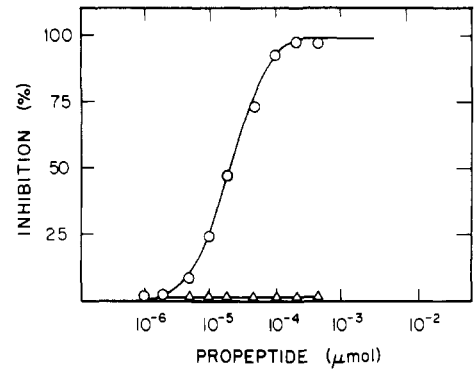

FIGURE 6: Inhibition radioimmune assay of type II procollagen. 50 μL of type II procollagen (5000 cpm) and 50 μL of antiserum to type II N propeptide were used. The antiserum was diluted 1:10. The second antibody was 500 μL of sheep anti-rabbit IgG. Other conditions are as described in the text. Type II N propeptide (○); type II C propeptide (△).

bacterial collagenase, and the digest was reduced and applied immediately to a 15% polyacrylamide gel. Prolonged exposure of the radioactive gel revealed only the presence of a single band migrating as the type II C propeptide; no N propeptide was seen (lane 4 in Figure 2). Type II procollagen was also prepared by incubating the cells without fetal calf serum for use as antigen in immunological studies.

Characterization of the N Propeptide with Antibodies. Antibodies were raised in rabbits by repeated intradermal injections of the purified N propeptide. Because of the poor response obtained with the N propeptide alone, the peptide was conjugated to hemocyanin to increase the immune response (Avrameas & Ternyck, 1969). Thereafter, high titers were obtained and injections of unconjugated N propeptide were used for booster injections.

The antisera against the N propeptide precipitated more than 90% of ^{14}C-labeled N propeptide (Figure 5). The same antisera precipitated more than 80% of ^{14}C-labeled type II procollagen. The amount precipitated was not increased by retitrating the equivalence point for precipitation of rabbit IgG with the second antibody. Failure to achieve 100% precipitation of ^{14}C-labeled procollagen was previously observed with antibodies against the type I C propeptide (Olsen et al., 1977). A similar observation was made with monoclonal antibodies to type I collagen (Linsenmayer et al., 1979). The antisera precipitated less than 15% of type I procollagen under the same conditions (Figure 5). It did not precipitate the C propeptide from type II procollagen. Also, the antisera did not precipitate type II procollagen that had been treated with pepsin to remove the N and C propeptides (Figure 5).

In further experiments, the antisera were used for inhibition radioimmune assays. As indicated in Figure 6, the isolated N propeptide, at a concentration of 10^{-7} M, completely inhibited precipitation of type II procollagen. As expected, the C propeptide from type II procollagen had no effect.

Discussion

This report presents the first isolation of type II N propeptide in amounts adequate for chemical and physical characterization. The peptide was isolated from the medium of sternal cartilages, a tissue which synthesizes predominantly type II collagen (Dehm & Prockop, 1973; Uitto, 1977; Linsenmayer et al., 1979). The size of the medium peptide was similar to that of N propeptide isolated from type II procollagen by cleavage with a specific procollagen N proteinase (Tuderman et al., 1978). Conclusive evidence that the medium peptide was in fact type II N propeptide was provided by the observation that antibodies to the propeptide specifically precipitated type II procollagen. The same antibodies did not precipitate type II procollagen after the propeptides were removed with pepsin and did not precipitate the C propeptide of type II procollagen.

Several structural and functional properties of the N propeptide were established here. The N propeptide clearly contains a collagen-like domain since it was digested by bacterial collagenase and had an amino acid composition similar to that of collagen. On the basis of the glycine content, as much as three-quarters of the peptide could consist of Gly-X-Y sequences. The CD spectrum further established that it had a collagen-like conformation. From the amino acid composition and CD spectrum, the N propeptide appeared to contain a globular domain similar to the N-terminal globular domain of N propeptides from proα1(I) and proα1(III) chains [for review, see Timpl & Glanville (1981)]. The size of the globular domain of the type II N propeptide was, however, considerably smaller. No collagenase-resistant fragment was obtained under conditions that provide a fragment of 77–86 residues from type I and type III N propeptides. Also, only two half-cysteine residues were present in the type II N propeptide whereas 10 were found in the type I N propeptide and 12 in the type III N propeptide. The type II N propeptide was about two-thirds the size of the type I and type III N propeptides as estimated by gel electrophoresis in $NaDodSO_4$. Preliminary analysis of the N propeptide by sedimentation equilibrium indicated that the trimer has a molecular weight of about 27 000 (S. Curran, P. Bruckner, and D. J. Prockop, unpublished data) and, therefore, the monomer a molecular weight of about 9000. This estimate for the monomer was about two-thirds the value of 15 000 daltons for type I and III N propeptides as determined by the same technique (Engel et al., 1977; Bruckner et al., 1978).

The data emphasize that the N propeptides of type I, II, and III procollagens all contain a collagen-like domain that forms a relatively stable triple helix. Although the amounts of the type II N propeptide available were not sufficient to establish the upper limits of the thermal transition at high concentration [see Engel et al. (1977)], the data demonstrated that the thermal transition at a low concentration was 40–45 °C. The disulfide-bonded trimer of the type III N propeptide has a thermal transition at 50–55 °C (Bruckner et al., 1978).

On the basis of the previous studies with procollagen N proteinase, it is likely that the two to eight residues that join the collagen-like domain to the α chain are similar in type I and type II procollagens, since the same enzyme cleaved both of the N propeptides from the remainder of the protein (Tuderman et al., 1978; Morikawa et al., 1980).

Although the collagen-like domain is an invariant feature of the N propeptides of type I, II, and III procollagens, the large globular domain of 77–88 residues is not. The type II N propeptide lacks a large portion of the globular domain to which several functions have been ascribed, including the suggestion that it acts as a feedback inhibitor for the synthesis of type I and type III procollagens but not type II (Paglia et al., 1979; Wiestner et al., 1979; Hörlein et al., 1981).

The antibodies obtained to the N propeptide are the first raised against type II procollagen. These antibodies will be useful in studying the metabolic fate of the N propeptide and locating it in cells and tissues. Also, they will be useful in studying the gene switching between the synthesis of type I and type II procollagen, which occurs during cell differentiation and dedifferentiation [see Bornstein & Sage (1980)]. The same antibodies may be useful in studying disease processes such as rheumatoid arthritis (Andriopoulos et al., 1976) and polychondritis (Foidart et al., 1978) in which antibodies to type II collagen appear to play a critical role.

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References

Andriopoulos, N. A., Mestecky, J., Miller, E. J., & Bennett, J. C. (1976) *Arthritis Rheum. 19*, 613–617.

Avrameas, S., & Ternyck, T. (1969) *Immunochemistry 6*, 43–52.

Becker, U., Helle, O., & Timpl, R. (1977) *FEBS Lett. 73*, 197–200.

Bonner, W. M., & Laskey, R. A. (1974) *Eur. J. Biochem. 46*, 83–88.

Bornstein, P., & Sage, H. (1980) *Annu. Rev. Biochem. 49*, 957–1003.

Brodsky-Doyle, B., Leonard, K. R., & Reid, K. R. M. (1976) *Biochem. J. 159*, 279.

Brown, F. R., III, Carver, J. P., & Blout, E. R. (1969) *J. Mol. Biol. 39*, 307.

Brown, F. R., III, Hopfinger, A. J., & Blout, E. R. (1972) *J. Mol. Biol. 63*, 85.

Bruckner, P., Bächinger, H. P., Timpl, R., & Engel, J. (1978) *Eur. J. Biochem. 90*, 595–603.

Curran, S., & Prockop, D. J. (1982) *Biochemistry* (in press).

Dehm, P., & Prockop, D. J. (1973) *Eur. J. Biochem. 35*, 159–166.

Engel, J., Bruckner, P., Becker, U., Timpl, R., & Rutschmann, B. (1977) *Biochemistry 16*, 4026–4033.

Epstein, E. H., Jr. (1974) *J. Biol. Chem. 249*, 3225–3231.

Foidart, J.-M., Abe, S., Martin, G. R., Zizic, T. M., Barnett, E. V., Lawley, T. J., & Katz, S. I. (1978) *N. Engl. J. Med. 299*, 1203–1207.

Gerard, S., Puett, D., & Mitchell, W. M. (1981) *Biochemistry 20*, 1857–1865.

Guzman, N. A., Graves, P. M., & Prockop, D. J. (1978) *Biochem. Biophys. Res. Commun. 84*, 691–698.

Hayashi, T., Curran-Patel, S., & Prockop, D. J. (1979) *Biochemistry 18*, 4182–4187.

Hoffmann, H. P., Olsen, B. R., Chen, H. T., & Prockop, D. J. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 4304–4308.

Hörlein, D., Fietzek, P. P., Wachter, E., Lapière, C. M., & Kuhn, K. (1979) *Eur. J. Biochem. 99*, 31–38.

Hörlein, D., McPherson, J., Goh, S. H., Bornstein, P. (1981) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 40*, 1213.

Laskey, R. A., & Mills, A. D. (1975) *Eur. J. Biochem. 56*, 335–341.

Linsenmayer, T. F., Hendrix, M. J. C., & Little, C. D. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 3703–3707.

Merry, A. H., Harwood, R., Woolley, D. E., Grant, M. E., & Jackson, D. S. (1976) *Biochem. Biophys. Res. Commun. 71*, 83–90.

Morikawa, T., Tuderman, L., & Prockop, D. J. (1980) *Biochemistry 19*, 2645–2650.

Morris, N. P., Fessler, L. I., & Fessler, J. H. (1979) *J. Biol. Chem. 254*, 11024.

Nist, C., von der Mark, K., Hay, E. D., Olsen, B. R., Bornstein, P., Ross, R., & Dehm, P. (1975) *J. Cell Biol. 65*, 75–87.

Olsen, B. R., Hoffmann, H.-P., & Prockop, D. J. (1976) *Arch. Biochem. Biophys. 175*, 341–350.

Olsen, B. R., Guzman, N. A., Engel, J., Condit, C., & Aase, S. (1977) *Biochemistry 16*, 3030–3037.

Paglia, L., Wilczek, J., Diaz de Leon, L., Martin, G. R., Hörlein, D., & Müller, P. K. (1979) *Biochemistry 18*, 5030–5034.

Pesciotta, D. M., Silkowitz, M. H., Fietzek, P. P., Graves, P. N., Berg, R. A., & Olsen, B. R. (1980) *Biochemistry 19*, 2447–2454.

Rohde, H., & Timpl, R. (1979) *Biochem. J. 179*, 643–647.

Smith, B. D., McKenney, K. H., & Lustberg, T. J. (1977) *Biochemistry 16*, 2980–2985.

Timpl, R., & Glanville, R. W. (1981) *Clin. Orthop. Relat. Res.* (in press).

Tuderman, L., Kivirikko, K. I., & Prockop, D. J. (1978) *Biochemistry 17*, 2948–2954.

Uitto, J. (1977) *Biochemistry 16*, 3421–3429.

Uitto, J., Hoffman, H.-P., & Prockop, D. J. (1977) *Arch. Biochem. Biophys. 179*, 654–662.

Wiestner, M., Krieg, T., Hörlein, D., Glanville, R. W., Fietzek, P. P., & Müller, P. K. (1979) *J. Biol. Chem. 254*, 7016–7023.