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Synthesis of [Pro α1(IV)]$_3$ Collagen Molecules by Cultured Embryo-Derived Parietal Yolk Sac Cells†

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ABSTRACT: Electron immunohistochemical studies demonstrate that cultured embryo-derived parietal yolk sac (ED-PYS) carcinoma cells synthesize type IV collagen. This material has been isolated and characterized. The collagen obtained after limited pepsin digestion from the medium in which the cells are grown is composed of homogeneous components with a molecular mass of ~95 000 daltons. When chromatographed on (carboxymethyl)cellulose under denaturing conditions, the chains elute as acidic components slightly before the human α1(I) chain and coincident with the position of elution of the pepsin-derived human α1(IV) chain. This analysis indicates the presence of a single type of collagen chain in the pepsin-derived ED-PYS synthesized material. In addition, the profile of cyanogen bromide (CNBr) cleavage products obtained from the pepsin-derived ED-PYS cell collagen chains is essentially identical with that derived from the human α1(IV) chain. Isolation of the medium collagen in the absence of pepsin digestion reveals the presence of two high molecular weight components equivalent in size to procollagen α chains. However, both high molecular weight products yield CNBr cleavage products that correspond to those obtained from the pepsin-derived α1(IV) chain. The ED-PYS cell-associated collagens obtained with or without the use of pepsin contain components that are essentially identical with those isolated from the culture-medium collagen. These data provide definitive evidence for the existence of type IV collagen molecules composed solely of α1(IV) procollagen chains and further document the usefulness of ED-PYS cells for investigating the biosynthesis of basement membrane components.

Basement membranes are the ubiquitous extracellular matrices found at the boundaries between cells and the connective tissue stroma (Martinez-Hernandez & Amenta, 1983). Significant evidence has accumulated indicating that these structures participate in a variety of physiological processes including filtration, structural support, and cell attachment (Hay, 1981; Kuehn et al., 1982) and that alterations in the functions, composition, and biosynthesis of the components that comprise basement membranes occur in a variety of pathological states (Martinez-Hernandez & Amenta, 1983).

Recent investigations have established that basement membranes are composed of several components with laminin (Timpl et al., 1979), entactin (Carlin et al., 1981), heparan sulfate proteoglycan (Kanwar & Farquhar, 1979), and type IV collagen (Bailey et al., 1979; Bornstein & Sage, 1980; Crouch & Bornstein, 1979; Dehm & Kefalides, 1978; Dixit & Kang, 1979; Gay & Miller, 1979; Glanville et al., 1979; Kresina & Miller, 1978; Mayne & Zethergren, 1980; Miller & Gay, 1982; Qian & Glanville, 1984; Robey & Martin, 1981; Sage et al., 1979; Timpl et al., 1978; Treub et al., 1982) being the best characterized of the constituents. To date, evidence indicates that the collagenous molecules isolated from these structures contain two genetically distinct types of collagen chains, designated α1(IV) and α2(IV) (Bornstein & Sage, 1980; Miller & Gay, 1982). However, the precise organization of these chains in any basement membrane remains unresolved with evidence having been presented for the existence of both homotrimeric (Dehm & Kefalides, 1978; Robey & Martin, 1981) and heterotrimeric (Mayne & Zettergren, 1980; Qian & Glanville, 1984; Treub et al., 1982) molecules. This lack of definition reflects in part both the proteolytic susceptibility of type IV molecules and the lack of suitable in vitro model systems to study the biosynthesis and deposition of the col-

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lagenous components in basement membranes. Previous work has indicated that the rat ED-PYS[1] tumor may be a suitable model system for the evaluation of basement membrane biosynthesis (Martinez-Hernandez et al., 1982). Therefore, the present study was undertaken to define the nature of the collagenous components synthesized by the cells of this tumor in culture.

Experimental Procedures

Materials. The sources of reagents for biochemical procedures and tissue culture have been previously detailed (Haralson et al., 1980). [^{3}H]Proline (sp act. = 108 Ci/mmol) was purchased from Amersham, and [^{3}H]glycine (sp act. = 44 Ci/mmol) was obtained from Schwarz/Mann. Human type I collagen was prepared as previously detailed (Kresina & Miller, 1979), and mouse type IV collagen was obtained from Bethesda Research Labs. The origin of the rat ED-PYS cell line as well as the clone used in these studies has been described (Martinez-Hernandez et al., 1982). Radioactive α1(V) chains employed as molecular size markers for fluorography were isolated from cultured Chinese hamster lung cells as previously detailed (Haralson et al., 1980).

Growth of Cells and Metabolic Labeling. ED-PYS cells were grown and maintained in Dulbecco's modified Eagle's minimal (DMEM) medium containing 10% fetal bovine serum. For immunohistochemical studies, the cells were grown on Tissue-Tek microchambers to the indicated state of confluency. To obtain radioactive collagen, the cells were grown to confluency ($\sim 3 \times 10^4$ cells/cm^2) in 150-mm dishes, and the medium was removed and replaced with 25 mL/dish DMEM medium supplemented with 50 μg/mL ascorbic acid and 10 μCi/mL [^{3}H]proline or [^{3}H]glycine. After incubation for 18 h, the culture medium was removed and clarified by centrifugation (3000 rpm for 30 min in a Beckman JA-10 rotor), and the supernatant fraction was adjusted to 10 mM *N*-ethylmaleimide (NEM) and 0.5 phenylmethanesulfonyl fluoride (PMSF). For the preparation of cell-associated collagens, the cell layers were scraped into 0.5 M HOAc ($\sim$8 mL/dish). The suspension was adjusted to the NEM and PMSF concentrations specified above, stirred for 24 h at 4 °C, and then clarified by centrifugation at 5000 rpm in a Beckman JA-10 rotor for 30 min. Either the clarified culture medium or the acid-soluble fraction of the cell layer was then used as the starting material for all subsequent procedures.

Electron Immunohistochemical Localization of Type IV Collagen. Mouse type IV collagen was injected into New Zealand white rabbits according to previously described procedures (Martinez-Hernandez et al., 1982). The IgG fraction was isolated by ammonium sulfate precipitation followed by chromatography on Affi-Blue Gel (Martinez-Hernandez et al., 1981). The antibody was further purified by affinity chromatography on a column containing ED-PYS laminin. The monospecificity of the antibody preparation was established by ELISA (Martinez-Hernandez et al., 1982), immunoblotting, and comparison with monospecific antibodies provided by Dr. G. R. Martin, N.I.D.R. (Mynderse et al., 1983). Subconfluent and confluent ED-PYS cultures were rinsed in cold phosphate-buffered saline, fixed in fresh 4% formaldehyde at 4 °C for 1 h and reacted with primary antibodies (antitype IV collagen, antitype I collagen, antitype III collagen, or normal rabbit serum), followed by goat anti-rabbit IgG and rabbit peroxidase–antiperoxidase complex as previously described (Martinez-Hernandez et al., 1981, 1982; Martinez-Hernandez, 1984; Mynderse et al., 1983;). Ultrathin selections were examined and photographed without further staining.

Isolation and Purification of ED-PYS Culture Medium and Cell Layer Collagen. The collagen present in the culture medium was isolated with or without the use of pepsin as previously detailed (Haralson et al., 1980, 1984). Similar procedures were performed on the acid-soluble fraction derived from ED-PYS cell layers to obtain the cell-associated collagen. The typical yields obtained by these protocols of [^{3}H]proline-labeled proteins per 150-mm culture dish were 1.62×10^6 cpm in untreated medium preparations, 4.83×10^5 cpm in pepsin-treated medium preparations, 4.89×10^6 cpm in untreated cell layer preparations, and 1.01×10^6 cpm in pepsin-treated cell layer preparations. The typical yield per 150-mm dish of pepsin-resistant [^{3}H]glycine-labeled proteins recovered from the culture medium was 8.1×10^5 cpm.

SDS–Polyacrylamide Gel Electrophoresis and Fluorography. Molecular weight analysis of the collagens or procollagens present in the various fractions was performed by SDS–polyacrylamide gel electrophoresis with the method of Laemmli (Laemmli, 1970). Samples were dissolved in sample buffer (62.5 mM Tris-HCl, pH 6.8, 2.5% SDS, 1.25% 2-mercaptoethanol, 12.5% glycerol, and 0.005% bromophenol blue), heated for 15 min at 85 °C, and then applied to a 12 × 15 cm polyacrylamide slab gel (separating gel), which was overlayed with a 1 × 15 cm 3% polyacrylamide gel (stacking gel). The samples were electrophoresed at 20 mA until the tracking dye entered the separating gel, and the current was then increased to 40 mA. Electrophoresis was continued until the tracking dye reached the bottom of the separating gel. The completed gels were prepared for fluorography by the method of Laskey & Mills (1975), and after the gel was dried at 80 °C under vacuum, the distribution of radioactive components was determined by exposing KODAK X-Omat AR film to the gel for 72 h at −70 °C. All gels contained radioactive CHL cell layer pepsin-derived collagen, which contains only α1(V) chains (Haralson et al., 1980); the position of migration of this chain was employed as a molecular weight standard for the fluorographs. The positions of migration of the α chains and β components of human type I collagen were determined in parallel gels after staining with Coomassie Brilliant Blue as previously detailed (Haralson et al., 1978) and were used for molecular weight estimates of the ED-PYS components.

Characterization of ED-PYS Cell Culture-Medium Collagen Chains. Pepsin-derived [^{3}H]glycine-labeled collagenous protein isolated from the medium of cultured ED-PYS cell was reduced, alkylated, and chromatographed in denatured form on a 1.2 × 155 cm column of agarose beads as previously described (Haralson et al., 1980). The components eluting from the agarose column in the position for type IV collagen α chains were combined, desalted, and then lyophilized. A portion of the purified chains was then chromatographed on CM-cellulose under denaturing conditions as previously detailed (Haralson et al., 1980). Elution of radioactive ED-PYS components was determined by assaying aliquots (0.5 mL) dissolved in Aquasol of each 3.75-mL fraction in a liquid scintillation counter.

Characterization of the CNBr Cleavage Products of ED-PYS Collagen. The CNBr cleavage products present in pepsin-derived and precursor forms of the ED-PYS culture medium collagen were assessed by a modification of the two-dimensional SDS–polyacrylamide gel electrophoresis procedure (Barsh et al., 1981). Prior to electrophoresis in the

[1] Abbreviations: ED-PYS, embryo-derived parietal yolk sac; SDS, sodium dodecyl sulfate; CNBr, cyanogen bromide; CHL, Chinese hamster lung; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

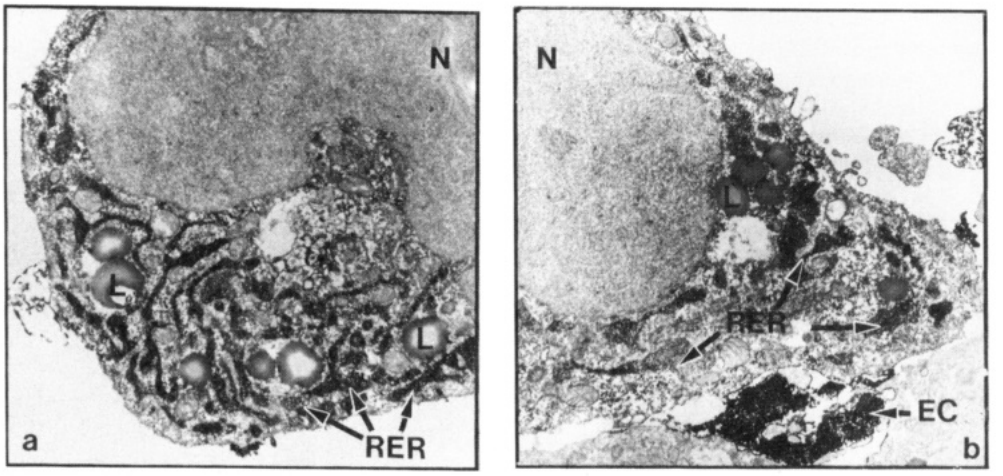

FIGURE 1: Electron immunohistochemical localization of type IV collagen in cultured ED-PYS cells. ED-PYS cells were grown in tissue culture, stained with antitype IV collagen antibodies, and processed for electron microscopic visualization as described under Experimental Procedures. (Panel A) ED-PYS cells in subconfluent culture. Portion of cell demonstrating that antitype IV collagen antibodies localize in the cisternae of the rough endoplasmic reticulum (RER). All other cellular compartments—nucleus (N), lipid vacuoles (L), and cytosol—are negative. No extracellular matrix is obvious in subconfluent cultures. Magnification 6035×. (Panel B) ED-PYS cells in confluent culture. Antitype IV collagen antibodies localize in the rough endoplasmic reticulum (RER) of confluent ED-PYS cells but not in the nucleus (N) or the lipid vacuoles (L). An abundant extracellular matrix (EC), which stains intensely with antitype IV collagen antibodies, has been deposited in the spaces between adjacent cells. Magnification 6888×.

first dimension, samples were subjected to reduction in 25% 2-mercaptoethanol (Adelstein & Kuehl, 1970) to facilitate CNBr cleavage of the type IV chains (Kresina & Miller, 1979). The samples were then electrophoresed on 5% SDS–polyacrylamide gels, and the proteins in the completed gels were then cleaved with CNBr as described (Barsh et al., 1981). These processed gels were then fused to the top of 10% SDS–polyacrylamide gels, and the combined gels were electrophoresed until the tracking dye was approximately 2 mm from the bottom of the gel. The profiles of the CNBr cleavage products in either the pepsin-derived ED-PYS chains or the metabolic precursors to the chains were then visualized by fluorography as described above.

RESULTS

Electron Immunohistochemical Location of Type IV Collagen in ED-PYS Cell Cultures. Previous evidence has indicated that antitype IV collagen antibodies react with the tumor matrix synthesized by ED-PYS carcinomas in rats (Martinez-Hernandez et al., 1982). In culture, ED-PYS carcinoma cells likewise reacted with antitype IV collagen antibodies (Figure 1). The nucleus and cytosol were negative, but the abundant cisternae of the rough endoplasmic reticulum were distended with the antigen. Subconfluent cultures displayed little extracellular matrix (Figure 1, panel A), but confluent cultures had an abundant extracellular matrix rich in type IV collagen (Figure 1, panel B). Normal rabbit serum, antitype I collagen, or antitype III collagen antibodies reacted with neither the cells nor the cell matrix (data not shown). Thus, by immunological criteria, cultured ED-PYS cells have maintained, as has been previously demonstrated in vivo (Martinez-Hernandez et al., 1982), the capacity to synthesize type IV collagen.

Characterization of ED-PYS Culture-Medium Collagen. Insight into the nature of the collagen synthesized by ED-PYS cells was obtained by assessing the size, chemical properties, and CNBr peptide profile of the pepsin-derived collagen secreted into the culture medium. As shown in Figure 2, lane A, the pepsin-resistant collagen obtained from the ED-PYS cell culture medium contains components that migrate after reduction approximately the same as human $\alpha 1$(I) chain and thus exhibit an apparent molecular weight of ~95 000.

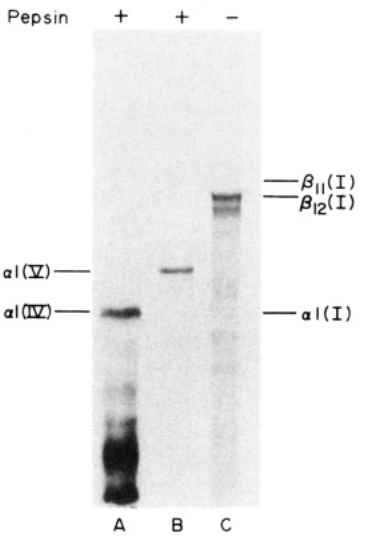

FIGURE 2: Fluorograph of SDS–polyacrylamide gel electrophoresis of ED-PYS cell culture-medium collagens. Samples of ED-PYS collagen labeled with [^{3}H]proline were isolated with (lane A) or without (lane C) pepsin and were electrophoresed on 5% SDS–polyacrylamide gels under reducing conditions, and fluorography was performed on the completed gels as described under Experimental Procedures. In this experiment, 100 000 cpm of pepsin-derived ED-PYS collagen and 100 000 cpm of native material were applied to lanes A and C, respectively. Pepsin-derived CHL cell layer collagen (60 000 cpm), which contains only $\alpha 1$(V) chains (Haralson et al., 1980), was applied in lane B and was used as an internal molecular weight marker for the fluorograph. The positions of migration of the $\alpha 1$ and β components from human type I collagen were determined in parallel gels as described under Experimental Procedures, and their positions of migration are indicated at the right side of the figure.

Without reduction, these preparations essentially remain at the top of 5% polyacrylamide gels (data not shown). These observations suggest that the pepsin-derived ED-PYS culture medium collagen is composed of a homogeneous set of subunits, which contain interchain disulfide bonds.

The pepsin-derived components that comprise the ED-PYS culture-medium collagen were isolated after reduction and alkylation by agarose chromatography as described under Experimental Procedures and were further characterized by denaturing CM-cellulose chromatography. As shown in Figure

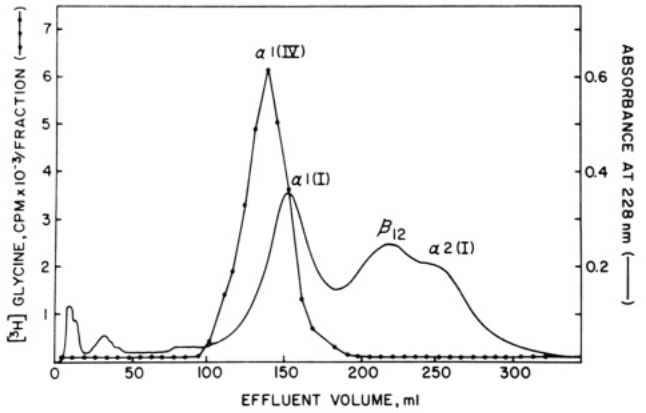

FIGURE 3: CM-cellulose chromatography of ED-PYS pepsin-derived culture-medium collagen chains. The sample (35 000 cpm of [^{3}H]-glycine-labeled pepsin-derived ED-PYS collagen components recovered after agarose chromatography) was chromatographed on CM-cellulose under denaturing conditions as previously detailed (Haralson et al., 1980). The elution of the human type I components (—) was continuously monitored at 228 nm, and the elution of the radioactive ED-PYS cell chains (●) was determined as described under Experimental Procedures. Recovery of applied radioactivity was 86%.

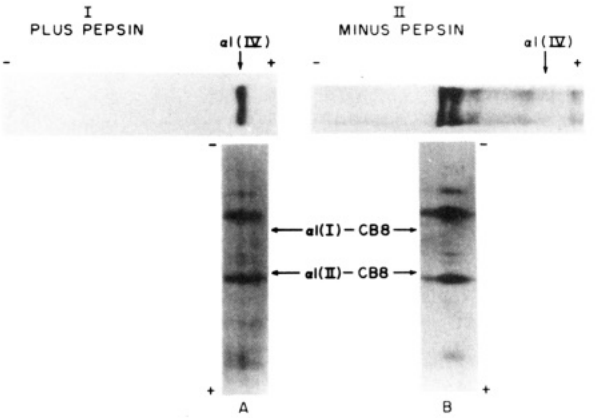

FIGURE 4: Composite fluorograph of two-dimensional SDS–polyacrylamide gel electrophoresis of CNBr peptides derived from ED-PYS cell culture-medium collagen chains. ED-PYS culture-medium collagens prepared with (I) or without (II) pepsin were reduced and electrophoresed on 5% SDS–polyacrylamide cylindrical gels. The proteins within the gels were then cleaved with CNBr, and the resulting peptides were resolved as described under Experimental Procedures. Lane A under (I) is the fluorograph of the peptides obtained from the ED-PYS pepsin-derived collagen, and lane B under (II) is the fluorograph of the peptides obtained from the ED-PYS culture-medium collagen prepared without pepsin. The positions of electrophoretic migration of the human collagen peptides α1(I)-CB8 and α1(II)-CB8 were determined as previously detailed (Kresina & Miller, 1979) and are indicated relative to the major CNBr cleavage products derived from the ED-PYS components.

3, the chains derived from this collagen homogeneously elute from the resin as relatively acidic components, slightly preceding the elution of the human α1(I) chain. This observation confirms the previous finding (Figure 2, lane A) that the collagen isolated by limited pepsin digestion from the culture medium of ED-PYS cells contains a single type of collagen chain. Furthermore, these chains elute from CM-cellulose in approximately the same position as the human α1(IV) chain (Gay & Miller, 1979; Kresina & Miller, 1979), a finding that provides initial insight into the chemical identity of these components and that is consistent with the immunohistochemical staining of the cells with antitype IV collagen antibodies (Figure 1).

Further evidence in support of the concept that the pepsin-derived collagen obtained from the culture medium of ED-PYS cells contains only α1(IV) chains was obtained by assessing the profile of the major CNBr-derived peptides of the ED-PYS cell pepsin-derived components. As shown in lane A of Figure 4, a profile of CNBr-derived peptides that resembles that obtained from the human α1(IV) chain (Kresina & Miller, 1979) was obtained upon cleavage of the pepsin-derived ED-PYS chains with CNBr. Additionally, this cleavage profile lacks any of the major CNBr-derived peptides that are indicative of the α2(IV) chain (Kresina & Miller, 1979). Thus, on the basis of the size of the pepsin-derived chains, their position of elution from CM-cellulose, and the profile of the major CNBr peptides derived from these chains, we conclude that the collagen secreted into the culture medium and recovered after pepsin treatment consists solely of molecules containing α1(IV) chains.

The usefulness of this cell line to study basement membrane biosynthesis requires a precise definition of the genetic types of collagen chains synthesized. Previous reports have demonstrated the proteolytic lability of molecules in the type IV group (Bailey et al., 1979; Crouch & Bronstein, 1979; Dixit & Kang, 1979; Gay & Miller, 1979; Glanville et al., 1979; Kresina & Miller, 1979; Mayne & Zettergren, 1980; Robey & Martin, 1981; Sage et al., 1979; Timpl et al., 1978; Treub e t al., 1982). Therefore, additional studies were undertaken to characterize the components contained in the native collagen molecues secreted by ED-PYS cells into the culture medium. As shown in Figure 2, lane C, the collagen molecules derived from the culture medium by differential salt fractionation but without pepsin treatment contain two components, which under reducing conditions migrate approximately the same distance as the β components of type I collagen and exhibit apparent molecular masses of ~180 000 and ~170 000 daltons, respectively. Thus, molecular-size analysis suggests that the native collagen molecules secreted by ED-PYS cells contain two distinct components that approximate in size the values reported for α1(IV) and α2(IV) procollagen chains. However, analysis by two-dimensional SDS–polyacrylamide gel electrophoresis of the CNBr cleavage products obtained from the two components (Figure 4, lane B) indicates a complement of peptides that corresponds to that of the pepsin-derived ED-PYS collagen chain (Figure 4, lane A). Furthermore, no additional major peptides that would be indicative of the α2(IV) collagen chain sequence are contained among the CNBr cleavage products of the two native components. As the data in this paper have documented that the pepsin-derived collagen secreted into the culture medium by ED-PYS cells contains only α1(IV) chains, the finding of a similar CNBr peptide profile for the native components indicates that they must both be related at the primary structure level to the α1(IV) collagen chain. The failure to detect additional CNBr peptides among the set derived from the native molecules may reflect either a reduced proline content in the CNBr peptides arising from the nonhelical sequence of the molecules or a large number of methionines within these sequences yielding small peptides; thus, either of these situations would yield peptides that would not be apparent by the method employed. Nevertheless, these observations demonstrate that the entire complement of collagen molecules synthesized by ED-PYS cells and secreted into the culture medium is composed of [pro α1(IV)]$_3$ collagen molecules.

Initial Characterization of ED-PYS Cell Layer Collagen. Previous studies have indicated that a differential distribution of collagen types may occur in cell culture with the complement of collagens isolated from the culture medium being disparate from that which remains associated with the cells (Haralson et al., 1980, 1984; Sage et al., 1981; Sasse et al.,

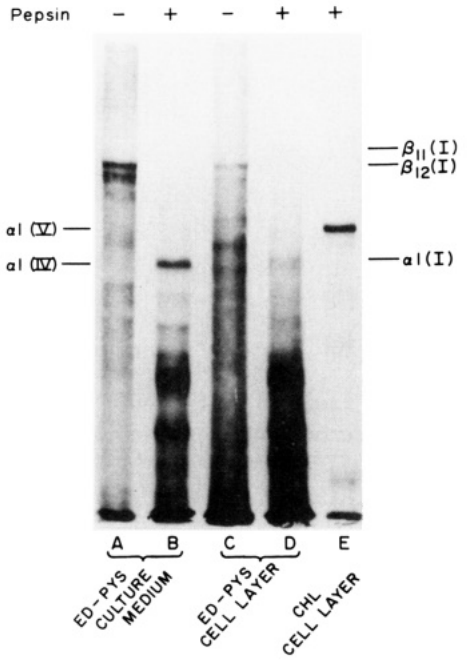

FIGURE 5: Fluorograph of SDS–polyacrylamide gel electrophoresis of ED-PYS culture-medium and cell-layer collagens. Samples of ED-PYS cell collagen from either the culture medium (lanes A and B) or the cell layer (lanes C and D) isolated without (lanes A and C) or with (lanes B and D) pepsin were electrophoresed on 5% SDS–polyacrylamide gels run under reducing conditions, and fluorography was performed on the completed gels as described under Experimental Procedures. In this experiment, the amounts of radioactive sample applied in lanes A–D were 110000, 100000, 240000, and 220000 cpm, respectively. CHL cell layer collagen (60000 cpm) was applied in lane E and used as an internal molecular weight standard for the fluorograph. The positions of migration of the human type I α1 chain and the β components were determined as described under Experimental Procedures and are indicated at the right side of the figure.

1981). Because of these precedents, studies were undertaken to initially assess the nature of the ED-PYS cell-associated collagens. As shown in Figure 5, lane D, the collagen recovered from ED-PYS cell layers following limited digestion with pepsin containing chains that under reducing conditions exhibit the same size as the α1(IV) chains derived with pepsin from the ED-PYS culture medium collagen (Figure 5, lane B). Similarly, the material isolated from the cell layer without the use of pepsin (Figure 5, lane C) contains components that are essentially the same size as those that comprise the non-pepsin-treated collagen obtained from the culture medium (Figure 5, lane A). Even though both preparations derived from ED-PYS cell layers contain a substantial amount of low molecular weight material, which reflects the increased protein content in the acid-soluble fraction of the cell layer relative to the culture medium, these data demonstrate that the number and size of the cell-associated ED-PYS collagenous components are essentially the same as those in the culture medium and that no additional predominant component is found in the cell-associated collagens. Therefore, it is concluded that essentially no differential distribution in collagen types between the culture medium and the cell layer occurs in this cell system and that the Ed-PYS cell-associated collagen contains components that likewise are related to the α1(IV) collagen chain at the primary structure level.

Discussion

The data in this paper provide definitive evidence that the collagen synthesized by cultured ED-PYS cells consists of molecules that are composed solely of α1(IV) collagen chains. This conclusion is based upon the common chemical features exhibited by the constituent chains present in the pepsin-derived ED-PYS culture-medium collagen and the pepsin-derived human α1(IV) chain. These common features include (1) an apparent molecular mass of 95000 daltons (Figure 2, lane A), (2) ion-exchange properties when chromatographed on CM-cellulose (Figure 3), and (3) similar size and number of CNBr cleavage products (Figure 4, lane A). Additionally, the collagenous protein derived from the culture medium without pepsin exhibits a profile of major CNBr peptides that is essentially identical with that obtained from the pepsin-derived chains (Figure 4, lane B). Finally, the chain composition of the cell-associated collagen synthesized by ED-PYS cells is essentially the same as that which is secreted into the culture medium (Figure 5). The fact that α1(IV) chains are recovered after pepsin digestion and selective salt precipitation (Figure 5, lanes B and D) indicates that these components must be organized in native molecules in both the culture medium and the cell layer. Therefore, on the basis of these findings, we conclude that the majority of the collagenous protein synthesized by cultured ED-PYS cells is initially produced as α1(IV) procollagen chains, which then form [pro α1(IV)]$_3$ collagen molecules.

Of additional interest is our finding by polyacrylamide gel electrophoresis of two apparently different proteins of procollagen chain size (Figure 2, lane C), both of which yield a complement of major CNBr fragments indicative of the pepsin-derived α1(IV) chain (Figure 4, lane B). Using the mouse teratocarcinoma cell line PYS-2, other investigators (Oberbaumer et al., 1982) have identified two type IV procollagen chains similar in size to those synthesized by ED-PYS cells (Figure 2, lane C); however, these investigators presented evidence that indicated that the two proteins represented different gene products [i.e., the α1(IV) and α2(IV) procollagen chains]. It should be noted that in their report it was observed that antibodies specific for the α1(IV) chain could not distinguish between two procollagen chain size products (see Figure 2; Oberbaumer et al., 1982), which suggests that the mouse PYS-2 cell line may also produce two components with different electrophoretic mobilities, both of which are related to the α1(IV) procollagen chain. It is clear, however, that in regard to collagen biosynthesis the rat ED-PYS cells are distinct from the mouse PYS-2 cells (Oberbaumer et al., 1982) in that no component that corresponds to the α2(IV) procollagen chain primary structure can be identified among the ED-PYS cell products (data in this paper).

Several possibilities exist to explain our finding of two ED-PYS cell synthesized procollagen chain size components that correspond at the primary structural level to the α1(IV) procollagen chain. First, the faster migrating material may be a partially processed form of the larger chain. However, in several preparations from both the culture medium and the cell layer, the observed relative amounts of the two high molecular weight components were essentially the same as shown in Figure 2, lane C. This observation is seemingly inconsistent with a processing mechanism being the explanation for the origin of the two high molecular weight products. Underhydroxylation of some of the α1(IV) procollagen chains appears to be an equally unlikely reason, given the constant ratio of both high molecular weight components in all preparations. A third possibility is that the two forms of α1(IV) procollagen chains represent the products of different alleles similar to the recent description of two α2(I) collagen chain sequences that are simultaneously expressed in one form of osteogenesis imperfecta (Byers et al., 1983). If this were the

case for type IV collagen synthesis in the ED-PYS system, it should be noted that such a putative difference in primary structure between the two products must occur in the nonhelical or pepsin-sensitive portion of the chains as treatment with this protease yields only one size class of molecules (Figure 5, lanes B and D). A fourth possibility to explain the origin of two α1(IV) procollagen chain sequences produced by ED-PYS cells is that they represent the same primary sequence that is differentially glycosylated. It is now established that cultured tumorogenic cells possess the capability to heterogeneously glycosylate identical protein sequences, and this in turn results in a differential mobility of the same primary sequence on SDS–polyacrylamide gels (Eipper & Mains, 1977; Hakomori & Kannagi, 1983; Hatton et al., 1983). Therefore, the two forms of α1(IV) procollagen chains may simply reflect differential glycosylation of the same primary sequence, but for the same reason iterated in the previous argument, this heterogeneity must occur in the nonhelical or pepsin-sensitive portions of the chains.

These investigations were initiated to further characterize a cloned cultureable cell line established from an embryo-derived parietal yolk sac tumor previously demonstrated to possess the capability to synthesize laminin in vivo (Martinez-Hernandez et al., 1982). The results of this work and the previous biochemical and immunological investigations establish this cell line as a unique system in that at least two of the major extracellular matrix components that are intrinsic to basement membranes, i.e., laminin and type IV collagen, are synthesized. However, in the ED-PYS system, the synthesis of type IV collagen is apparently restricted to the α1(IV) procollagen chain sequence, which results in the formation of homotrimeric procollagen molecules. Furthermore, the growth properties of this clone [growth to high cell density, rapid generation time ($\sim$16 h at 37 °C) and high plating efficiency (>90%)] when considered in the context of the biosynthetic capacity of these cells documented in this paper indicate that this cell line will be of potential value in further delineating the molecular events involved in the biosynthesis of basement membrane components. In addition, this cell line offers a unique source for isolating the mRNA that codes for the rat α1(IV) procollagen chain sequence, and such preparations will be used for the construction of cDNA molecules to investigate the regulation of type IV procollagen biosynthesis in normal and pathological conditions.

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