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A Collagen-like Glycoprotein of the Extracellular Matrix Is the Undegraded Form of Type VI Collagen†

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ABSTRACT: The 140 000-dalton collagenous glycoprotein (CGP) from calf aorta and ligament characterized by Gibson & Cleary (1982) [Gibson, M. A., & Cleary, E. G. (1982) *Biochem. Biophys. Res. Commun. 105*, 1288–1295] has been studied. In the electron microscope, rotary-shadowed CGP molecules appear similar to the dimers of type VI collagen (short-chain collagen, intima collagen) described by other authors [Furthmayr, H., Wiedemann, H., Timpl, R., Odermatt, E., & Engel, J. (1983) *Biochem. J. 211*, 303–311] except that they have larger globular domains. As shown by gel electrophoresis, pepsin treatment of CGP at 4 °C either before or after reduction releases polypeptide chains corresponding in size to those of type VI collagen. Electron microscopic examination shows that pepsin digestion of nonreduced CGP removes the outer globular domains, reduces the size of the inner ones, and separates the paired central strands. The residual structures look like type VI collagen dimers. When intact CGP is reduced, monomers with two large globular ends are obtained. Pepsin digestion of monomers removes most or all of both globular domains. In immunoblots, CGP and its pepsin-derived fragments react with antibodies directed against type VI collagen. The results indicate that type VI collagen is an integral component of CGP.

Up until now, short-chain collagen or intima collagen, a component of the extracellular matrix isolated primarily from human or bovine placenta, has been characterized only after pepsin digestion (Furuto & Miller, 1980, 1981; Jander et al., 1981, 1983; Odermatt et al., 1983). As it represents a new and unique collagen, different from other collagen types, the term "type VI collagen" has recently been proposed (Furthmayr et al., 1983; Jander et al., 1983). Amino acid analysis

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revealed that most of the molecule is collagenous in nature, whereas one-fourth of it is comprised of a noncollagenous, glycoproteinaceous part. Nothing is known regarding the biosynthesis of the putative precursor or in vivo functional forms of this kind of collagen.

Recently, a structural model of intima collagen based on electron microscopic studies has been proposed (Furthmayr et al., 1983). The monomer is believed to consist of a 105-nm-long collagen triple helix with a large globular domain at one end and a smaller globular unit at the other. Dimers are composed of two monomers arranged in a staggered antiparallel fashion. The authors also described the occurrence of higher order polymers and filamentous structures. In light of the evidence, it is possible that type VI collagen in its natural undegraded state is part of a more complex molecule.

An intriguing component of the extracellular matrix is the glycoprotein recently extracted from fetal calf aorta and ligament without the use of proteases by Gibson & Cleary (1982). This glycoprotein has a molecular mass of 140 000 daltons after reduction and is partly of collagenous character. Since it seemed possible that the glycoprotein might be related to type VI collagen, we compared the two with respect to their chemical characteristics, immunological behavior, and electron microscopic appearance. Of special interest was whether the glycoprotein could be converted to type VI collagen by protease treatment. The present study presents data which suggest that its collagenous part is identical with type VI collagen. In keeping with the designation of Gibson and Cleary, we call it provisionally "collagen-like glycoprotein" (CGP).[1]

Materials and Methods

Protein Isolation. Procedures for the isolation and purification of type VI collagen have been reported in detail elsewhere (Jander et al., 1983).

Isolation and purification of CGP followed the procedure described by Gibson & Cleary (1982) except that the collagenase treatment was omitted. Calf aorta or ligamentum nuchae obtained from the slaughterhouse was homogenized and extracted successively with solutions of 0.9% saline, 0.5 M KCl, 6 M guanidine hydrochloride, and 6 M guanidine hydrochloride with 0.05 M dithioerythritol; the supernatants corresponded to the extracts S, SK, SKG, and SKGD, respectively, of Gibson and Cleary. Each extraction solution also contained a cocktail of protease inhibitors (0.1 mM phenylmethanesulfonyl fluoride, 20 mM EDTA, 1 mM *p*-(chloromercuri)benzoate, and 10 mM *N*-ethylmaleimide) and was buffered to pH 7.5 with 0.05 M Tris-HCl. The extractions were allowed to proceed at 4 °C for 24 h. The supernatants were dialyzed against water and lyophilized.

The lyophilized extracts SKG and SKGD of calf aorta were extracted further with 0.05 M Tris, pH 7.5, at 37 °C, and both the supernatants and residues were reduced with mercaptoethanol and alkylated with vinylpyridine as described elsewhere (Jander et al., 1981). Ion-exchange chromatography was carried out on DEAE-cellulose (DE 52, Whatman) in 6 M urea and 0.05 M Tris, pH 8.3, at 20 °C. Elution was performed with a salt gradient. Fractions containing CGP were rechromatographed on Sephacryl S-400 (Pharmacia). The elution buffer was 6 M urea in 0.05 M Tris, pH 7.8. Further details of the chromatographic procedures are given in the figure legends.

[1] Abbreviations: EDTA, ethylenediaminetetraacetic acid; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; DEAE, diethylaminoethyl; $NaDodSO_4$, sodium dodecyl sulfate; DTE, dithioerythritol; CGP, collagen-like glycoprotein.

The extract SKG of calf ligament was applied directly to a Sephacryl S-400 column. The material which appeared in the exclusion volume was subsequently chromatographed on DEAE-cellulose under the same conditions as described above.

Analytical Methods. Amino acid analysis and electrophoresis on $NaDodSO_4$–polyacrylamide slab gels were performed as previously described (Jander et al., 1983).

Immunological Methods. Antibodies against the pepsinized, shortened form of bovine type VI collagen (short-chain collagen, SCC*) raised in rabbits according to Jander et al. (1981) were kindly provided by B. Voss. Immunoblotting of $NaDodSO_4$–polyacrylamide gels on nitrocellulose paper was adapted from Towbin et al. (1979).

Proteolytic Digestion. (*a*) *Bacterial Collagenase.* Samples were suspended in 0.025 M Tris with 0.01 M $CaCl_2$, 1 mM phenylmethanesulfonyl fluoride, and 5 mM *N*-ethylmaleimide, pH 7.4 (0.5 mg/0.25 mL), incubated with 25 units of collagenase (type III, Advanced Biofactures) at 37 °C for 2 h, acidified by addition of acetic acid, and lyophilized. Controls were treated in the same way, but without addition of enzyme. Under these conditions, collagen types I and V, but not the noncollagenous proteins fibronectin and albumin, were completely digested by collagenase.

(*b*) *Pepsin.* Samples were suspended in 0.5 M formic acid (1 mg/mL) and incubated with pepsin (Worthington) at an enzyme:substrate ratio of 1:10 at 4 °C for 24 h. In other experiments, the protein was first reduced and alkylated under nondenaturing conditions before being treated with pepsin as described already (Jander et al., 1981). To stop further digestion, samples were prepared immediately for electron microscopy or freeze-dried.

Electron Microscopy. Protein suspensions in glycerol were sprayed on to mica sheets with an air brush and rotary shadowed with platinum–carbon at an angle of about 5° followed by pure carbon as described by Tyler & Branton (1980). The replicas were placed on uncoated grids and photographed in a Philips EM 201 electron microscope.

Results

Isolation and Purification of Reduced and Alkylated CGP. The SKG and SKGD extracts of calf aorta contained CGP as a major glycoprotein which prior to reduction occurred as a high molecular mass disulfide-bonded component. Treatment of these extracts with 0.05 M Tris, pH 7.5, at 37 °C removed more readily soluble proteins including most of the type I collagen present, but a small amount of CGP was also found in the supernatant. After reduction and alkylation, CGP bound to a DEAE-cellulose column. It was eluted by means of a salt gradient (Figure 1A). The fraction containing CGP was freed from contaminants of higher and lower molecular mass in a subsequent gel chromatography step on Sephacryl S-400 (Figure 1B). An electrophoretogram of the purified protein, hereafter called "reduced and alkylated CGP", is shown in Figure 3, lane 4. CGP appeared as a single band or doublet, depending on the preparation, and stained strongly with periodic acid–Schiff reagent, indicating that it is a glycoprotein (not shown). No significant differences in electrophoretic pattern or collagenase sensitivity (see below) could be detected for samples from the different sources (i.e., SKG, SKGD, or supernatant and residue of Tris extraction).

Isolation and Purification of Nonreduced CGP. In extract SKG of calf nuchal ligament, the total amount of protein was less than in the corresponding aorta extract, but the band representing CGP appeared enriched as compared to the other bands (not shown). Therefore, this material seemed suitable for isolation of CGP without prior reduction of disulfide bonds.

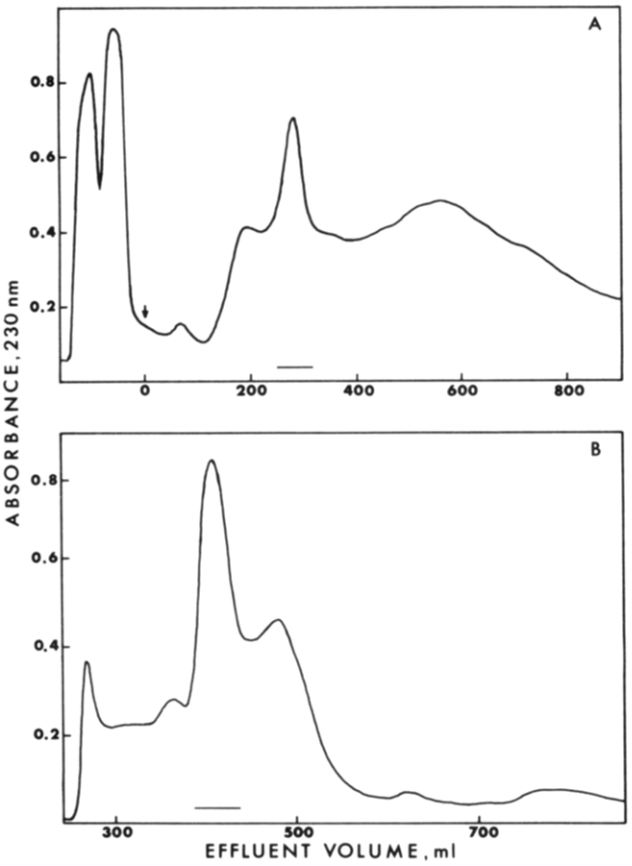

FIGURE 1: Chromatographic purification of reduced and alkylated CGP. (A) 100 mg of reduced and alkylated SKG extract from calf aorta was applied to a DEAE-cellulose column (2.5 × 14 cm) at 20 °C and subsequently eluted with 6 M urea in 0.05 M Tris, pH 8.3, and a salt gradient (0–0.25 M NaCl) over a total volume of 1000 mL. The flow rate was 120 mL/h. The arrow marks the start of the gradient. (B) 45 mg of the material in the fraction marked by a bar in (A) was rechromatographed on a Sephacryl S-400 column (2.5 × 145 cm) at room temperature. Elution was performed with 6 M urea in 0.05 M Tris, pH 7.8, at a flow rate of 35 mL/h. The peak marked by the bar contained purified reduced and alkylated CGP.

About half of the total lyophilized extract was soluble in 6 M urea in 0.05 M Tris, pH 7.8, and was chromatographed on Sephacryl S-400 at room temperature. Along with other species, CGP appeared as a protein of high molecular mass in the exclusion volume of the column (Figure 2A). Contaminant collagenous proteins were not retained during DEAE-cellulose chromatography, whereas CGP was bound to the column. The glycoprotein was eluted as a broad peak by the salt gradient (Figure 2B). Aliquots were taken from different fractions of the effluent and examined in the electron microscope. The main fraction (marked in Figure 2B), which appeared to contain few contaminants, was used for further study and will be referred to as "nonreduced CGP".

Electrophoretograms of this protein are shown in Figure 3, lanes 2 and 3. In addition to the main 140K band (here appearing as a doublet), four of five fainter bands in the range of 170 000–250 000 daltons arose after reduction; they were found in all fractions after DEAE-cellulose chromatography. Two minor bands of low molecular mass could not be removed by gel or ion-exchange chromatography.

Collagenase Treatment. Reduced and alkylated CGP was sensitive to bacterial collagenase (Figure 3, lane 7). After 2 h of digestion at 37 °C, the original 140K band had almost disappeared, and several new peptides became visible in gel electrophoresis. Under the same conditions, nonreduced CGP, in contrast, was not susceptible to collagenase (not shown).

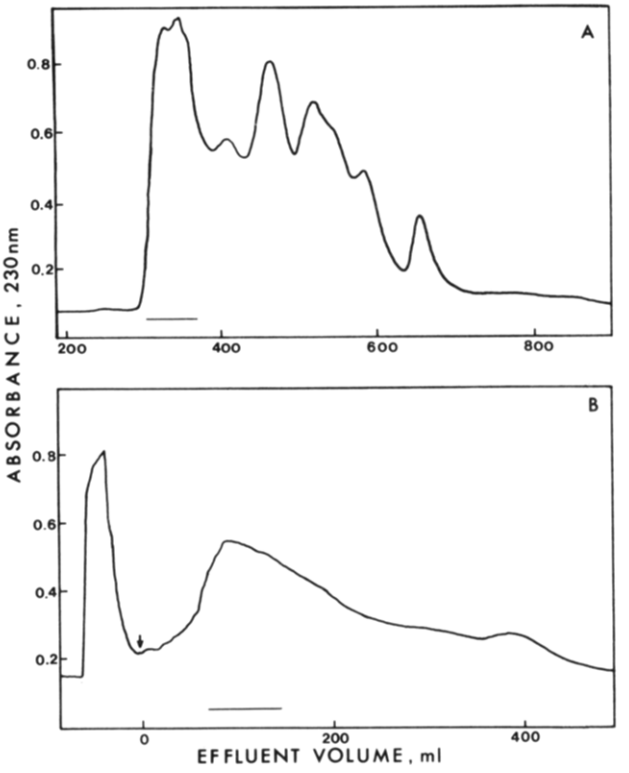

FIGURE 2: Chromatographic purification of nonreduced CGP. (A) 100 mg of extract SKG from calf ligament was chromatographed on a Sephacryl S-400 column (2.5 × 145 cm) and eluted as described in the legend for Figure 1B. (B) 50 mg of the material eluting in the exclusion volume marked by the bar in (A) was chromatographed on a DEAE-cellulose column (1.5 × 10 cm) at 20 °C. Elution was as described in the legend to Figure 1A except that the total volume was 600 mL and the flow rate 80 mL/h. The arrow marks the start of the gradient. The fraction marked by the bar contained purified nonreduced CGP.

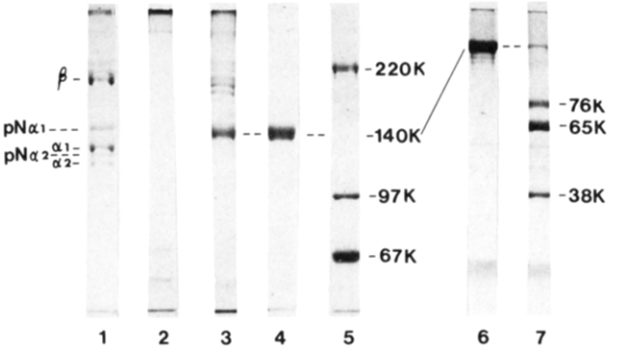

FIGURE 3: $NaDodSO_4$ gel electrophoresis of CGP performed on slab gels with an acrylamide concentration of 6% (lanes 1–5) and 10% (lanes 6 and 7). Lane 1, molecular mass standards of type I collagen and procollagen (pN from dermatosparactic calf skin); lane 2, nonreduced CGP (from calf ligament); lane 3, same material as in lane 2, reduced with DTE prior to electrophoresis; lane 4, reduced and alkylated CGP (from calf aorta); lane 5, globular protein molecular mass (in daltons) standards: fibronectin (220 000), phosphorylase *b* (97 400), bovine serum albumin (67 000); lanes 6 and 7, reduced and alkylated CGP after 2-h incubation without (lane 6) and with (lane 7) bacterial collagenase as described under Materials and Methods. Molecular masses of the three major digestion products were determined by comparison with globular protein standards.

Amino Acid Analysis. The amino acid compositions of reduced and alkylated CGP and nonreduced CGP are given in Table I. There is one obvious difference between the two forms: the content of aspartic and glutamic acids is markedly increased in the reduced and alkylated protein, whereas the amount of proline is considerably lower. The amino acid

Table I: Amino Acid Composition of Nonreduced CGP (SKG Extract from Calf Ligament), Reduced and Alkylated CGP (SKG Extract from Calf Aorta), and Type VI Collagen (from Bovine Placenta)[a]

amino acid	nonreduced CGP	reduced, alkylated CGP	type VI collagen
4-hydroxyproline	28	24	48
aspartic acid	94	120	86
threonine	41	43	29
serine	61	63	41
glutamic acid	114	132	101
proline	70	50	83
glycine	164	165	244
alanine	72	83	53
cysteine	11	10	18
valine	55	53	35
methionine	13	14	9
isoleucine	39	34	32
leucine	70	61	43
tyrosine	23	18	17
phenylalanine	33	29	21
hydroxylysine	10	14	44
lysine	38	32	32
histidine	10	10	5
arginine	54	45	59

[a] Results are given as residues per 1000 residues. Cysteine was determined as pyridylethylcysteine.

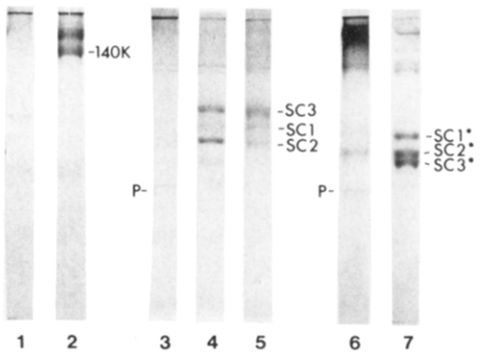

FIGURE 4: $NaDodSO_4$ gel electrophoresis of pepsin-derived fragments of CGP on 10% acrylamide slab gels. Samples in lanes 2, 4, and 5 were reduced with DTE prior to electrophoresis. P = pepsin. Lanes 1 and 2, nonreduced CGP; lanes 3 and 4, same material as in lanes 1 and 2 after digestion with pepsin; lane 5, bovine SCC (type VI collagen); lane 6, nonreduced CGP after reduction under nondenaturing conditions and pepsin digestion; lane 7, bovine SCC*. For details of pepsin treatment, see Materials and Methods, and for designation of polypeptide chains of type VI collagen, refer to Jander et al. (1983).

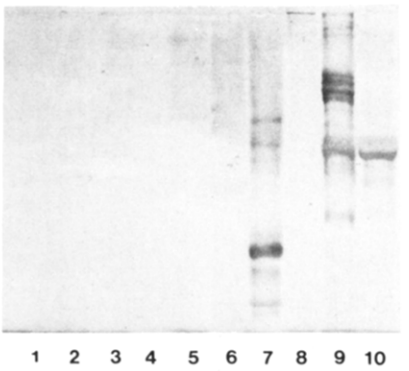

FIGURE 5: Immunoblot of different collagen types and CGP. A 10-μg of sample of each protein was loaded on each lane, and after electrophoresis on a 6% gel, the samples were electrophoretically transferred to nitrocellulose paper and incubated with anti-SCC* antiserum. The samples were the following: lane 1, pN-collagen (I); lane 2, type I collagen; lane 3, type III collagen; lane 4, type IV collagen; lane 5, type V collagen; lane 6, 7S collagen; lane 7, type VI collagen; lanes 8 and 9, nonreduced CGP; lane 10, reduced and alkylated CGP. Samples in lanes 3, 4, 6, 7, and 9 were reduced with DTE prior to electrophoresis.

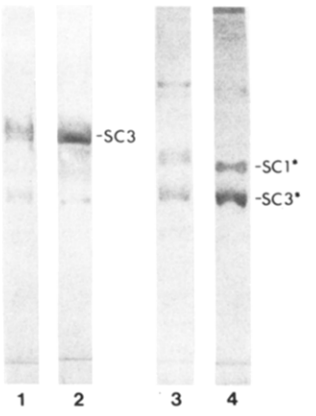

FIGURE 6: Immunoblot of the pepsin-derived fragments of CGP. After electrophoresis on a 10% gel, samples were electrophoretically transferred to nitrocellulose paper and incubated with anti-SCC* antiserum. Samples in lanes 1 and 2 were reduced with DTE before electrophoresis. Lane 1, nonreduced CGP after digestion with pepsin; lane 2, bovine SCC (type VI collagen); lane 3, nonreduced CGP after reduction under nondenaturing conditions and pepsin digestion; lane 4, bovine SCC*. Samples correspond to those in lanes 4–7 of Figure 4. The positions of the various chains were determined by comparison with a reference blot stained with Amido Black.

composition of type VI collagen is given for comparison. Note that the ratio of hydroxyproline to hydroxylysine is about 1:1 in type VI collagen, but not in either form of CGP.

Pepsin Treatment. Pepsin digestion of nonreduced CGP was carried out to determine whether a collagenous fragment could be obtained that shared some characteristics with type VI collagen. Figure 4 shows the electrophoretic pattern before (lanes 1 and 2) and after (lanes 3 and 4) digestion. After pepsin treatment, the main 140K and the higher molecular mass bands are absent. After reduction, the main bands of the protein correspond in size to the chains of type VI collagen [i.e., SC3, SC1, and SC2; cf. lane 5 and see Jander et al. (1983)].

In a previous paper (Jander et al., 1983), we demonstrated that type VI collagen (short-chain collagen, SCC), which had been reduced and alkylated in the native state, could be converted to its basic collagenous unit (SCC*) by a second pepsin treatment; SCC* lacks the glycoproteinaceous part of type VI collagen. When we applied this method to nonreduced CGP, we obtained similar bands but of slightly different mobility compared to the corresponding products from type VI collagen (Figure 4, lanes 6 and 7). The bulk of the protein, however, comprises high molecular mass aggregates that barely penetrate the gel.

Immunoblotting. As shown in Figure 5 (lanes 8–10), nonreduced as well as reduced and alkylated CGP reacted with antibodies against SCC*. In both blots, bands of lower molecular mass are also stained. Since these bands cannot be visualized in gel electrophoresis after Coomassie Blue staining, they are assumed to be degradation products that are present only at low concentration. The antiserum was directed specifically against type VI collagen (land 7) and CGP; it did not react with other collagen types (lanes 1–6). After incubation with anti-SCC*, the pepsin-derived fragments of CGP and type VI collagen displayed very similar staining patterns (Figure 6); SC3 and SC3* represent the bands that reacted most strongly.

Electron Microscopy. Rotary-shadowed molecules of native CGP are comprised of three rodlike and four globular elements in linear array (Figure 7A). A long central rodlike structure

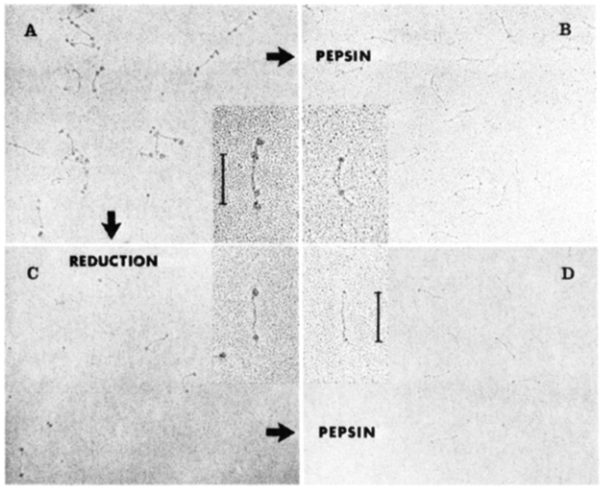

FIGURE 7: Rotary-shadowed nonreduced CGP: (A) untreated; (B) after pepsin digestion (cf. type VI collagen, Figure 8A); (C) after reduction and alkylation; (D) following reduction and alkylation and subsequent pepsin digestion. Magnification 31500× (insets, 67500×; bars represent 100 nm).

is situated between two globular domains. Extending from each globular domain is a thinner shorter rodlike element which terminates in another globular unit. The distance between the outer globule and the center of the farthest inner domain is about 105 nm.

Pepsin treatment alters the form of the CGP molecule (Figure 7B). Following incubation with pepsin, the outer globular domains are lacking, and the inner ones are much reduced in size. The medial rodlike element is often splayed into two parallel strands. The overall length of the molecule excluding the terminal globules remains unchanged, indicating that pepsin does not attack the rodlike elements. Pepsin-digested CGP molecules are structurally identical with the dimers of type VI collagen (cf. Figure 8A) described by Furthmayr et al. (1983).

The CGP molecules shown in Figure 7A are dimers. As in type VI collagen, two monomers are aligned in staggered antiparallel fashion in the CGP dimer, their rodlike elements overlapping in the central region of the molecule. Occasional images of CGP showing the terminal globule of a monomer (i.e., the inner globular domain of the dimer) pulled away from the opposing monomer provide support for this interpretation (Figure 8B,C). Although the dimer is the most frequent structure in CGP preparations, aggregations consisting of dimers linked together at their ends are found frequently (Figure 8C–F).

When CGP is reduced and alkylated, a heterogeneous collection of aggregated material is obtained (not shown), but one predominant species in the preparations is obviously the monomer of CGP (Figure 7C). The monomer is a 105-nm-long strand with two large terminal globular units. When reduced and alkylated material is digested with pepsin, both globular domains of the individual monomers are reduced in size (Figure 7D). Neglecting aggregates, molecules with two globular ends probably represent the most frequent form of the monomer after pepsin treatment. However, many monomers display only one or no globular domain. After longer pepsin treatment, no globular ends are found.

Discussion

We have investigated the 140 000-dalton collagenous glycoprotein originally isolated by Gibson & Cleary (1982) from calf aorta and ligament. This glycoprotein, here termed CGP, is similar to the detergent-insoluble glycoprotein of the extracellular matrix, GP140, characterized by Carter (1982a,b). Both share common chemical features: molecular masses of 140 000 daltons after reduction of disulfide bonds, occurrence of the amino acids hydroxyproline and hydroxylysine which are characteristic of collagenous sequences, and sensitivity to treatment with bacterial collagenase. Also common to both proteins are their low solubilities in nonionic detergents: the protein we isolated shows little tendency to dissolve in neutral buffers containing NP40 (R. Jander, unpublished results). Thus, CGP and GP140 probably represent the same glycoprotein. As discussed elsewhere (Carter, 1982b), CGP may also be the same or at least related to the collagenous glycoprotein MFP I secreted by cultured nuchal ligament cells (Sear et al., 1981). Another glycoprotein of the detergent-insoluble matrix described by Lehto (1983) has similar properties but is reported to be insensitive to bacterial collagenase.

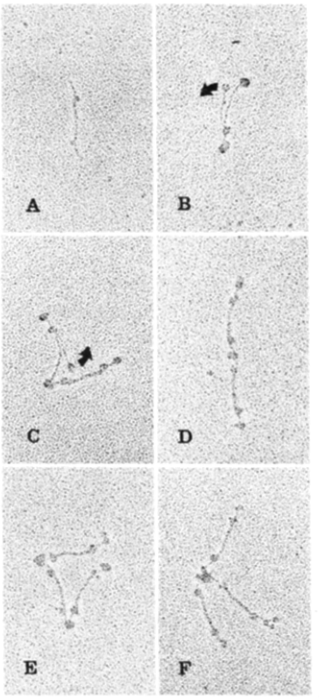

FIGURE 8: Rotary-shadowed type VI collagen and CGP molecules. (A) Type VI collagen dimer obtained by pepsin digestion of tissue. (B,C) Nonreduced CGP showing dimers in which the head of one monomer (arrows) is dissociated from the rodlike region of the adjacent antiparallel monomer. (D–F) Aggregates of nonreduced CGP produced by end to end association of individual dimers. Magnification 75000×.

For comparative purposes, CGP was isolated both with and without prior reduction of disulfide bonds. Nonreduced CGP can be distinguished from reduced and alkylated CGP on the basis of its lower content of acidic amino acids. This feature may be due to the presence of additional components in the nonreduced form of the protein. Both forms of CGP bound to DEAE-cellulose, although this deviates from the report of Gibson & Cleary (1982). Usually collagenous material is not retained by this ion-exchange material under the chosen conditions, but retardation of native types V and VI collagen has been reported (Abedin et al., 1982).

The estimated molecular mass of 140 000 daltons for the predominant protein band in gel electrophoresis was derived by comparison with globular protein standards. According to collagen standards, the protein band would correspond to M_r 100 000–110 000. Since CGP contains collagenous as well as noncollagenous domains, a precise molecular mass is difficult to assign from these data. Why this 140 000-dalton polypeptide chain was resolved in gel electrophoresis either

as a single band or sometimes as a doublet after both isolation procedures is not clear. Differences in the primary structure of the polypeptide chains or posttranslational modifications resulting in varying sugar content might be responsible.

Nonreduced CGP is insensitive to bacterial collagenase, whereas the reduced form is degraded to several peptides when treated with this protease. This finding indicates that disulfide bridges stabilize CGP, protecting it from collagenase attack. Compared to type I collagen, however, reduced CGP represents a poor substrate for collagenase, because longer reaction times and higher enzyme concentrations are required for complete degradation (not shown). A similar conclusion was drawn by Carter (1982b) regarding GP140 isolated from the detergent-insoluble matrix of cultured fibroblasts.

Since we suspected that CGP and type VI collagen are closely related, we digested guanidine-extracted CGP with pepsin with the expectation that a protein corresponding to type VI collagen might be released, because pepsin digestion is used to isolate type VI collagen from tissue. In fact, the high molecular mass fragment obtained dissociates after reduction into polypeptide chains of molecular masses corresponding to those of the chains SC1, SC2, and SC3 of type VI collagen described by Jander et al. (1983). After reduction under nondenaturing conditions, the fragment obtained by pepsin treatment likewise dissociates into three chains similar to those of the shortened form (SCC*) of type VI collagen. These results suggest that the molecular structure of CGP strongly resembles that of type VI collagen. Not in accord with this premise is the relative amount of hydroxyproline to hydroxylysine. Since these amino acids would have to be contained in the collagenous portion of the glycoprotein and since they occur in a 1:1 ratio in type VI collagen, the same proportion would be expected in CGP. We cannot presently reconcile this result, but we note that Gibson & Cleary (1982) did find a 1:1 ratio.

CGP is clearly related to type VI collagen immunologically, because both the reduced and nonreduced glycoproteins reacted with antisera directed against the shortened form of type VI collagen. We interpret the minor bands of molecular mass higher than the 140 000-dalton main component found in reduced electrophoretograms of the nonreduced glycoprotein to be aggregates of CGP, because these also reacted with anti-type VI collagen antiserum. However, one cannot rule out the possibility that other components of the extracellular matrix may have complexed to CGP, thereby increasing its apparent molecular mass. Such an explanation was proposed by Carter (1982b) with reference to GP140 from the detergent-insoluble matrix. Furthermore, the chains obtained from pepsin-treated nonreduced CGP after reduction under nondenaturing conditions react with anti-SCC* antiserum. Since this antiserum is specific for the collagenous region of type VI collagen and does not cross-react with other collagen types, the pepsin-derived fragments of CGP and type VI collagen contain identical determinants. Considering the structural and immunological data, type VI collagen seems to represent the collagenous part of CGP.

Viewed with the electron microscope, preparations of pepsin-treated guanidine-extracted CGP are seen to be degraded to molecules identical in appearance with dimers of type VI collagen. CGP molecules can be transformed to monomers by reduction of disulfide bonds, and subsequent pepsin digestion produces structures which look like the rodlike elements of type VI collagen monomers. Obviously, one dimer of type VI collagen is integrated into each dimer of CGP. Although the globular domains are visibly affected by the treatments just mentioned, no detectable change in the lengths of the molecules is incurred. Therefore, it is likely that the type VI collagen molecule represents essentially all of the collagenous moiety of the CGP molecule. The monomers of CGP are evidently held together in the dimer at least in part by disulfide bridges at their globular ends and by pepsin-sensitive structures in the central region of the molecule.

The location of CGP in tissue has not been directly established. However, it probably resides in the extracellular matrix, because each molecule contains a molecule of type VI collagen which is a known matrix component of blood vessel walls (Rauterberg et al., 1982). Furthermore, we could extract more than twice as much CGP from aorta by weight than type VI collagen, so the glycoprotein is evidently not a collagen-processing intermediate; such an intermediate would be expected to occur in substantially smaller quantities than its processed counterpart.

Little is known about the assembly and the biological function of CGP in the extracellular matrix. Carter (1982a) suggested that GP140, which may be the same as CGP, represents a non-membrane-bound matrix component involved in cell attachment and spreading, and Sear et al. (1981) proposed that the related glycoprotein MFP I is a component of elastin-associated microfibrils. The dimer of CGP could be a basic unit of fibrillar structures, because it is remarkably stable even in highly concentrated denaturing solvents such as guanidine hydrochloride and urea. Although Furthmayr et al. (1983) have postulated that type VI collagen molecules become cross-linked within their terminal rodlike parts in a scissorlike fashion, CGP dimers visualized in the electron microscope tend to aggregate at their globular domains. Whether this is artifactual or whether these molecules are so linked in the matrix remains to be determined. However, the pronounced tendency for the individual dimers to aggregate end to end would be an important prerequisite for the production of fibrillar structures.

We conclude that the collagen-like glycoprotein is the undegraded form of type VI collagen. The native length of the polypeptide chains is ostensibly retained throughout the extraction due to the protease inhibitors used. Sear et al. (1981) demonstrated that nascent MFP I from fibroblast cultures contains polypeptide chains of comparable length. Nevertheless, to what extent the denaturing conditions of extraction affect the conformation of the protein is unclear. As a nondegraded form of type VI collagen, CGP would be expected to contain three different polypeptide chains. However, after reduction, electrophoresis reveals only one band or a pair of bands. Therefore, whether the 140K component represents a single or several polypeptide chains which are not resolvable by gel electrophoresis due to similar molecular masses still remains to be established.

With respect to its partly collagenous, partly glycoproteinaceous composition, CGP resembles the enzyme acetylcholinesterase whose tail subunit contains both collagen-like and non-collagen-like domains. The pepsin-derived fragment of the tail subunit is a M_r 72 000, disulfide-bonded collagenous component that probably anchors the enzyme in the basement membrane (Rosenberry et al., 1982). Viewed as the undegraded form of type VI collagen, CGP would occupy an intermediate position between the "genuine" collagens of interstices and basement membranes and the structural glycoproteins.

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Monoclonal Antibodies against the *lac* Carrier Protein from *Escherichia coli*. 1. Functional Studies†

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ABSTRACT: The effects of various monoclonal antibodies against purified *lac* carrier protein on carrier-mediated lactose transport were studied in right-side-out membrane vesicles and in proteoliposomes reconstituted with purified *lac* carrier protein. Out of more than 60 monoclonal antibodies tested, only one antibody, designated 4B1, inhibits transport. Furthermore, the nature of the inhibition is highly specific in that the antibody inhibits only those transport reactions that involve net proton translocation (i.e., active transport, carrier-mediated influx and efflux under nonenergized conditions, and lactone-induced proton influx). In contrast, the antibody has little effect on equilibrium exchange and no effect on generation of the proton electrochemical gradient or on the ability of the carrier to bind a high-affinity ligand. Clearly, therefore, the antibody alters the relationship between lactose and proton translocation at the level of the *lac* carrier protein. When entrance counterflow is studied with external [1-^{14}C]lactose at saturating and subsaturating concentrations, it is apparent that antibody 4B1 mimics the effects of deuterium oxide [Viitanen, P., Garcia, M. L., Foster, D. L., Kaczorowski, G. J., & Kaback, H. R. (1983) *Biochemistry 22*, 2531]. That is, the antibody has no effect on the rate or extent of counterflow when external lactose is saturating but stimulates the efficiency of counterflow when external lactose is below the apparent K_m. It seems likely, therefore, that the antibody either inhibits the rate of deprotonation or alters the equilibrium between protonated and deprotonated forms of the carrier. Monovalent Fab fragments prepared from antibody 4B1 inhibit transport in a manner that is similar qualitatively to that of the intact antibody. However, intact 4B1 is approximately twice as effective as the Fab fragments on a molar basis, suggesting that the intact molecule binds bivalently while the Fab fragments bind 1:1. Support for this conclusion is provided by binding experiments with radiolabeled 4B1 and 4B1 Fab fragments presented in the following paper [Herzlinger, D., Viitanen, P., Carrasco, N., & Kaback, H. R. (1984) *Biochemistry* (following paper in this issue)].

β-Galactoside transport across the plasma membrane of *Escherichia coli* is mediated by the *lac* carrier protein (i.e., *lac* permease), an intrinsic protein encoded by the *lac y* gene [cf. Kaback (1983) for a recent review]. The protein catalyzes the coupled translocation of substrate with protons in a symport (cotransport) reaction. Thus, in the presence of a proton electrochemical gradient ($\Delta\bar{\mu}_{H^+}$,[1] interior negative and alkaline), downhill transport of protons in response to $\Delta\bar{\mu}_{H^+}$ drives uphill transport of substrate (i.e., active transport). Alternatively, downhill transport of substrate under nonenergized conditions drives the uphill transport of protons with generation of $\Delta\bar{\mu}_{H^+}$.

By use of a strain of *E. coli* with multiple copies of the *lac y* gene, a highly specific photoaffinity label for the *lac* carrier protein, and reconstitution of transport activity in proteoliposomes, the *lac* carrier protein has been purified to homogeneity (Newman et al., 1981; Foster et al., 1982). Proteoliposomes reconstituted with this single polypeptide species catalyze all of the transport activities observed in right-side-out

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[1] Abbreviations: $\Delta\bar{\mu}_{H^+}$, the proton electrochemical gradient; RSO, right-side-out; octyl glucoside, octyl β-D-glucopyranoside; $\Delta\psi$, membrane potential; PMS, phenazine methosulfate; *p*CMBS, *p*-(chloromercuri)-benzenesulfonate; NPG, *p*-nitrophenyl α-D-galactopyranoside.

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