A study of the interaction *in vitro* between type I collagen and a small dermatan sulphate proteoglycan

Niels ULDBJERG* and Carl Christian DANIELSEN†

*Gynecology/Obstetrics Research Laboratory, Bartholin Building, and †Department of Connective Tissue Biology, Institute of Anatomy, University of Aarhus, DK-8000 Århus C, Denmark

The interaction between a small dermatan sulphate proteoglycan isolated from human uterine cervix and collagen type I from human and rat skin was investigated by collagen-fibrillogenesis experiments. Collagen fibrillogenesis was initiated by elevation of temperature and pH after addition of proteoglycan, chondroitinase-digested proteoglycan or isolated side chains, and monitored by turbidimetry. Collagen-associated and unbound proteoglycan was determined by enzyme-linked immunosorbent assay after aggregation was complete. (1) The binding of proteoglycan to collagen could be explained by the presence of two mutually non-interacting binding sites, with $K_{a1} = 1.3 \times 10^8 \text{ M}^{-1}$ and $K_{a2} = 1.3 \times 10^6 \text{ M}^{-1}$. The number of binding sites per tropocollagen molecule was $n_1 = 0.11$ and $n_2 = 1.1$. The 0.1 high-affinity binding site per tropocollagen molecule indicates that the strong interaction between proteoglycan and collagen results from a concerted action of tropocollagen molecules in fibrils. Digestion of the proteoglycan with chondroitinase ABC did not affect these binding characteristics. (2) Proteoglycan did not affect the rate of fibrillogenesis, but increased the steady-state A_{400} by up to 90 %. This increase was directly proportional to the saturation of the high-affinity type of binding sites. Neither isolated core protein nor isolated side chains induced a similar high increase in steady-state A_{400} . (3) Electron micrographs showed that the fibril diameter was affected only to a minor extent, if at all, by the proteoglycan, whereas bundles of laterally aligned fibrils were common in the presence of proteoglycan. (4) Results obtained with human and rat collagen were similar.

INTRODUCTION

Small dermatan sulphate proteoglycans with M_r less than 100 000 have been isolated from several tissues (Cöster & Fransson, 1981; Uldbjerg *et al.*, 1983b; Franzén & Heinegård, 1984; Vogel & Heinegård, 1985). They have core proteins with apparent M_r of about 48 000 after chondroitinase ABC digestion, and only one dermatan sulphate side chain (Krusius & Ruoslahti, 1986). Iduronic acid constitutes 50–80 % of the uronic acid component. The physiological roles of the small dermatan sulphate proteoglycans are unknown, but there are indications of an interaction with collagen: electron-microscopic examinations have demonstrated that dermatan sulphate proteoglycans cover the surfaces of the collagen fibrils, with no intra-fibrillar proteoglycans being present, whereas it has been suggested that chondroitin sulphate proteoglycans are localized between the collagen fibrils (Scott, 1980; Scott & Orford, 1981; Haigh & Scott, 1986). The dermatan sulphate proteoglycans are localized almost exclusively at the *d* band, in the gap zone of the collagen fibril. Also Gallagher *et al.* (1983) found that the iduronic acid content of the dermatan sulphate proteoglycans may be crucial for the interaction with collagen. They cultured human skin fibroblasts on collagen gels and found that a dermatan sulphate proteoglycan enriched in iduronic acid bound specifically to the collagen fibres, whereas another dermatan sulphate proteoglycan that was enriched in

glucuronic acid accumulated in the culture medium. It remains to be shown if this interaction between some dermatan sulphate proteoglycans and collagen fibrils is mediated by the protein core, the glycosaminoglycans, the oligosaccharides, or combinations of these. Öbrink (1973), however, showed that dermatan sulphate side chains bind strongly to collagen, whereas chondroitin sulphate shows a low affinity for collagen.

In the present study we have examined the binding *in vitro* of a small dermatan sulphate proteoglycan isolated from the human uterine cervix to collagen type I, and we have investigated the effect of the dermatan sulphate proteoglycan on the collagen fibrillogenesis.

EXPERIMENTAL

Materials

Papain (twice crystallized; 16–40 BAEE units/mg) and chondroitinase ABC were products of Sigma Chemical Co., St. Louis, MO, U.S.A. Bovine serum albumin (fraction V) came from Calbiochem, Richmond, CA, U.S.A., DEAE-cellulose (DE-52) from Whatman Biochemicals, Maidstone, Kent, U.K., and Kenacid Blue R from BDH Chemicals, Poole, Dorset, U.K.

Dermatan sulphate proteoglycan

Proteoglycan was extracted from human uterine cervix with 4 M-guanidinium chloride and purified by density-

gradient centrifugation followed by DEAE-cellulose and Sepharose chromatography, as described previously by Uldbjerg *et al.* (1983a,b), who also described the degradation procedures. This proteoglycan is polydisperse, with an M_r of about 100 000. The protein core is monodisperse, with an apparent M_r of 47 000 after chondroitinase ABC digestion, and high contents of aspartic acid, glutamic acid and leucine. The dermatan sulphate moiety has a co-polymeric structure, with approximately equal quantities of iduronic acid- and glucuronic acid-containing disaccharides. Core protein was obtained by chondroitinase ABC digestion of the proteoglycan. Oligosaccharides formed by this treatment were not removed from the solution. Dermatan sulphate side chains were isolated by chromatography of papain-digested proteoglycan on a DEAE-cellulose column and quantified by hexosamine determination. The purities of the intact collagen and the core protein were monitored by SDS/polyacrylamide-gel electrophoresis on 8%-acrylamide gels (Neville, 1971).

Collagen type I

Acid-soluble collagen was obtained from the dorsal skin of 60-day-old male Wistar rats and from the abdominal skin of a 20-year-old male human who died suddenly without a prior period of disease or drug use. Extraction and purification by repeated precipitations of acetic acid-extracted collagen were performed as described previously (Danielsen, 1981). The rat skin collagen was freeze-dried and stored in liquid N_2 . The human skin collagen was prepared just before the experiments. The collagen preparations dissolved in 5 mM-acetic acid were filtered through a 0.8 μ m-pore-size membrane filter and stored at -20°C until the experiments were conducted. Samples from these solutions were taken for hydroxyproline determination (Danielsen *et al.*, 1986). When the experiments were commenced, the collagen solutions were thawed at 4°C , diluted with 5 mM-acetic acid and kept in an ice bath.

Enzyme-linked immunosorbent assay (ELISA)

Samples (1 mg) of the dermatan sulphate proteoglycan suspended in 0.9% (w/v) NaCl were used for immunization of rabbits, initially together with Freund's complete adjuvant and then for two booster injections (500 μ g) with Freund's incomplete adjuvant. The animals were bled twice monthly, starting 1 month after the first booster. Sera were not purified further.

Enzyme-linked immunosorbent assay was performed by the procedure of Engvall & Perlman (1971), with some modifications as described by Heinegård *et al.* (1985). The difference between A_{405} and A_{620} was determined after 1.5 h at room temperature on an ImmunoReader NJ-2000 (InterMed). Samples were assayed in quadruplicate, and concentrations were determined with a spline program (Reinsch, 1967) on a Hewlett-Packard 85 micro-computer.

Binding of proteoglycan to collagen

The proteoglycan was dissolved in double-concentrated PBS-Tw (0.27 M-NaCl/0.006 M-KCl/0.16 M- Na_2HPO_4 /0.002 M- KH_2PO_4 , pH 7.35, containing 0.10% Tween-20, used as substrate buffer in the ELISA) with 5 μ l of 1 M-NaOH/ml to neutralize the acetic acid of the collagen solution, and mixed with equal volumes of collagen (200 μ g/ml) in 5 mM-acetic acid, incubated at

30°C for 2 h for fibrillogenesis, and centrifuged at 10 000 g for 10 min. The pellets were washed, re-centrifuged and dissolved in 5 mM-acetic acid (2 h at room temperature) and, to prevent renewed fibrillogenesis, heated at 60°C for 5 min before mixing with equal volumes of double-concentrated PBS-Tw with 5 μ l of 1 M-NaOH/ml. Proteoglycans were quantified in these redissolved pellets as well as in the supernatants by the ELISA.

Fibrillogenesis assay

The substance to be tested was dissolved in 0.2 M-NaCl/0.1 M-Tris/HCl, pH 7.4, to which was added 5 μ l of 1 M-NaOH/ml to neutralize the acetic acid of the collagen solution and stored on ice for up to 1 month. Dilutions of this solution were mixed with equal volumes of collagen (200 μ g/ml) and de-gassed in a tube on ice. The cold mixture was transferred to a preheated cuvette placed in a Cary 118 spectrophotometer with a water-jacketed cuvette chamber equilibrated to 30°C . Fibril formation was followed by recording the A_{400} .

Hydroxyproline assay

At 4 h after fibrillogenesis had been initiated in a tube under conditions described above for the fibrillogenesis assay, the tube was centrifuged at 10 000 g for 10 min, and hydroxyproline was determined (Stegeman & Stalder, 1967) in the pellet and in the supernatant after hydrolysis in 6 M-HCl at 100°C for 17 h.

Electron microscopy

At 24 h after fibrillogenesis had been initiated in a tube under conditions described above for the fibrillogenesis assay, fibrils were placed on carbon-coated Formvar films supported on copper grids (Anderson *et al.*, 1977). The fibrils were negatively stained with 1% (w/v)

Fig. 1. Binding of dermatan sulphate proteoglycan from human uterine cervix to skin collagen type I (100 μ g/ml)

Rat collagen and proteoglycan ($\blacksquare$), rat collagen and chondroitinase-ABC-digested proteoglycan ($\blacktriangle$) and human collagen and proteoglycan ($\bullet$) were mixed before initiation of heat aggregation by elevation of temperature to 30°C and pH to 7.4 for 2 h, followed by centrifugation. An ELISA was used to quantify proteoglycan in the pellet (collagen-bound DS-PG) and in the supernatant (free DS-PG). Bars indicate S.E.M. ($n = 4$).

Fig. 2. Rosenthal plot of data obtained with rat skin collagen type I and dermatan sulphate proteoglycan from the human uterine cervix given in Fig. 1.

The M_r of tropocollagen was taken as 300 000 and that of the proteoglycan (DS-PG) as 100 000. A non-linear least-squares curve-fitting procedure for a model with two types of mutually non-interacting binding sites (Ottolenghi & Jensen, 1983; Jensen *et al.*, 1984) was used (—). The two lines S_1 (-----) and S_2 (·····) represent each type of binding sites. The slopes of the lines are equal to the intrinsic affinity constants, $K_{a1} = 1.3 \times 10^8 \text{ M}^{-1}$ and $K_{a2} = 1.3 \times 10^6 \text{ M}^{-1}$, and the intercepts at the abscissa give the numbers of binding sites, $n_1 = 0.11$ and $n_2 = 1.1$. All concentrations in the Figure are molar.

phosphotungstic acid adjusted to pH 7.4 with NaOH for approx. 10 min, and then air-dried. The fibrils were examined in a JEOL Jem 100S electron microscope operated at 80 kV.

RESULTS

The binding of human cervical dermatan sulphate proteoglycan to rat skin collagen type I is shown in Fig. 1. The Rosenthal (1967) plot shows that the binding of proteoglycan to collagen could be explained by the presence of two types of mutually non-interacting binding sites with different affinities (Fig. 2). The affinity constant of the high-affinity binding sites (K_{a1}) was $1.3 \times 10^8 \text{ M}^{-1}$, and the number of binding sites (n_1) was 0.11 per tropocollagen molecule. Corresponding values for the low-affinity binding sites were $K_{a2} = 1.3 \times 10^6 \text{ M}^{-1}$ and $n_2 = 1.1$ per tropocollagen molecule.

The binding to rat skin collagen of the core protein isolated by chondroitinase ABC digestion of the proteoglycan was similar to that obtained with the intact proteoglycan within the concentration range tested (Fig. 1).

The results obtained with human skin collagen type I were similar to those obtained with the rat skin collagen type I within the concentration range tested (Fig. 1).

Fig. 3 shows how proteoglycan affected the fibrillogenesis of rat skin collagen type I (100 $\mu\text{g}/\text{ml}$) as monitored by turbidimetry. A steady-state A_{400} was obtained after 2–3 h. The same applied when proteo-

Fig. 3. Effect of intact or altered dermatan sulphate proteoglycan (DS-PG) of human uterine cervix on fibrillogenesis of rat skin collagen type I

A 100 μg portion of collagen and 5 μg of DS-PG, or 5 μg of chondroitinase-digested DS-PG (core protein) or 3.5 μg of side chain were combined in 1 ml of 0.1 M-NaCl/0.05 M-Tris/HCl, pH 7.4. The A_{400} was monitored during incubation at 30 °C.

Fig. 4. Relative increase in steady-state A_{400} above control as a function of the concentration of cervical dermatan sulphate proteoglycan present during the fibrillogenesis of rat skin collagen type I (100 $\mu\text{g}/\text{ml}$)

Fibrillogenesis was initiated by elevation of temperature to 30 °C and pH to 7.4. Turbidity was monitored, and steady-state A_{400} was determined after 3 h (●). The saturations of high-affinity (-----) and low-affinity (·····) binding sites were calculated by using the constants given in Fig. 2.

glycan was present. Steady-state A_{400} was proportional to the collagen concentration (50–200 $\mu\text{g}/\text{ml}$), and the rate of fibrillogenesis was somewhat increased at the higher collagen concentrations (results not shown). In the presence of proteoglycan, the rate of fibrillogenesis

Fig. 5. Electron micrographs of collagen fibrils reconstituted in the absence or in the presence of dermatan sulphate proteoglycan

Fibrils of purified type I collagen from rat skin (a, b, e, f) and human skin (c, d) are shown. The fibrils were reconstituted in the absence (a, c, e) or in the presence (b, d, f) of 5 μ g of dermatan sulphate proteoglycan per

was not changed sufficiently, whereas the steady-state A_{400} was increased. Fig. 4 gives the relative increase in steady-state A_{400} as a function of proteoglycan concentration. This increase is directly proportional to the binding of proteoglycan to the high-affinity binding sites as indicated by the curved line. The maximum increase in steady-state A_{400} was 90%. When present in equivalent concentrations, neither isolated dermatan sulphate side chains nor chondroitinase-ABC-digested proteoglycan gave a similar high increase in A_{400} to that with intact proteoglycan (Fig. 3). Dextran T-40 (5 μ g/ml) and albumin (5 μ g/ml) did not change the fibrillogenesis curve significantly (results not shown).

The rate of fibrillogenesis obtained with human skin collagen type I did not differ from that obtained with rat skin collagen. Furthermore, 5 μ g of proteoglycan/ml induced a relative increase in A_{400} of 80%, which was similar to that obtained with rat skin collagen type I.

Less than 3% of the hydroxyproline could be detected in the supernatants 4 h after fibrillogenesis of rat skin collagen type I had been initiated. This percentage was the same for controls and samples containing 5 μ g of proteoglycan/ml. Thus the higher steady-state A_{400} that developed in the presence of proteoglycan was not related to enhanced incorporation of collagen into fibrils. Therefore, electron-microscopic examinations were performed to study if an alteration in fibril organization could explain this phenomenon.

Electron micrographs showed collagen fibrils with typical native cross-striation (Fig. 5). Compared with the control fibrils, some characteristic features of the fibrils formed in the presence of dermatan sulphate were apparent. The fibrils appeared more densely packed, and thick bundles of fibrils were present. At first glance, these bundles looked like thick fibrils. By investigating at high magnification ($\times 63000$) and by studying these bundles in the electron microscope, the bundles were found to consist of laterally aligned discrete fibrils, their light and dark bands being in register. The mean ($\pm$ S.D., n) diameter of the individual fibrils was estimated as 119 (± 25 , 15) nm and 146 (± 36 , 15) nm for those formed in the absence and presence of dermatan sulphate proteoglycan respectively. The difference in fibril diameters for the two samples was more pronounced at lower magnifications. Difficulties in discerning the discrete fibrils for those formed in the presence of the dermatan sulphate proteoglycan, and a less marked lateral demarcation of the more faintly stained control fibrils, may account for the minor difference in fibril diameter that was found at the high magnification. Thus the dermatan sulphate proteoglycan influences the interaction between the collagen fibrils rather than the fibril thickness.

DISCUSSION

Vogel *et al.* (1984) have isolated a small dermatan sulphate proteoglycan from bovine tendon. This proteoglycan shared many biochemical characteristics with the proteoglycan which we have isolated from human uterine

100 μ g of collagen by elevating the pH to 7.4 and the temperature to 30 $^{\circ}$ C; 24 h after the initiation of fibrillogenesis, fibrils were placed on grids and stained with 1% (w/v) phosphotungstic acid (pH 7.4).

cervix and used in the present study. The two proteoglycans had similar sizes, as evaluated by gel chromatography, and both core proteins migrated on SDS/polyacrylamide-gel electrophoresis with an apparent M_r of 48 000 after chondroitinase ABC digestion. Vogel *et al.* (1984), however, demonstrated that their small proteoglycan produced a marked inhibition of the rate of fibrillogenesis, and that the steady-state A_{400} became only slightly increased, when a phosphate/saline buffer without Tes was used. They also demonstrated that apparently rather similar small bovine proteoglycans from aorta and cartilage that have different protein cores (Heinegård *et al.*, 1985) induced an up to 50 % increase in the steady-state A_{400} and only an insignificant change in the rate of fibrillogenesis. These results were somewhat changed when Tes was included in the buffer. Then the aorta and the cartilage proteoglycan did not affect the fibrillogenesis at all, whereas the retardation induced by the tendon proteoglycan became even more pronounced. This retardation could at least partly be explained by the formation of thinner collagen fibrils (Vogel & Trotter, 1987). Scott *et al.* (1986) have isolated a bovine skin proteoglycan and found this to delay collagen fibrillogenesis in a way similar to that by the tendon proteoglycan, as described above. Thus there might be two populations of small dermatan sulphate proteoglycans: one population, including the tendon and the skin proteoglycan, that mainly delays the fibrillogenesis of collagen type I, and another population, including the cervix and the aorta proteoglycan, that mainly increases the steady-state A_{400} . Differences in protein cores might explain these differences (Heinegård *et al.*, 1985).

The 0.1 high-affinity binding site per tropocollagen molecule found in the present work (Fig. 2) is consistent with a specific interaction between collagen fibrils and dermatan sulphate proteoglycan ($K_{a1} = 1.3 \times 10^8 \text{ M}^{-1}$), and not between tropocollagen molecules and the proteoglycan. The equimolar binding ($n_2 = 1.1$) of proteoglycan to low-affinity binding sites suggests a low-affinity interaction between the proteoglycan and the individual tropocollagen molecules ($K_{a2} = 1.3 \times 10^6 \text{ M}^{-1}$).

In the human uterine cervix, the dermatan sulphate proteoglycans constitute by weight 1–2 % of the collagen (Uldbjerg *et al.*, 1983b), a value that gives about 30 % saturation of the high-affinity binding sites *in vitro* and about 30 % increase in steady-state A_{400} (Fig. 4). According to Scott & Orford (1981), the dermatan sulphate proteoglycans cover the surfaces of the collagen fibrils, with no intrafibrillar proteoglycans being present. From this point of view, the number of high-affinity binding sites per tropocollagen molecule varies according to the fibril diameter *in vivo*.

Protein cores from some large proteoglycans (Oegema *et al.*, 1975) and also isolated dermatan sulphate (Öbrink, 1973) have been shown to interact with collagen. Furthermore, Cöster *et al.* (1981) have demonstrated self-association of a small dermatan sulphate proteoglycan from bovine sclera as the apparent M_r decreased when associative conditions were changed to dissociative conditions. This self-association was mediated by the dermatan sulphate side chains. In the present study we have demonstrated that the binding of the cervix proteoglycan to rat skin collagen was unaffected by chondroitinase ABC digestion. Core protein, dermatan sulphate side chains, albumin and Dextran T-40 did not have a similar effect on steady-state A_{400} to that of the

intact proteoglycan, and it is therefore suggested that induction of lateral alignment of the collagen fibrils, as demonstrated by electron microscopy, is not a characteristic shared by all macromolecules, but is related to some intact dermatan sulphate proteoglycans, including those isolated from the cervix and aorta. Scott *et al.* (1986) have performed electron-microscopy examinations and found *d*-periodic arrangement of glycosaminoglycans on the surfaces of collagen fibrils formed in the presence of bovine skin dermatan sulphate proteoglycan, whereas isolated dermatan sulphate chains did not bind to the fibrils. Thus it seems reasonable to hypothesize that these proteoglycans bind to collagen fibrils via the protein cores/oligosaccharides and associate through the dermatan sulphate side chains with proteoglycans at other collagen fibrils, thus facilitating the lateral alignment demonstrated in this study. If so, not only differences in protein cores but also differences in dermatan sulphate side chains can explain the different effect of different proteoglycans on collagen fibrillogenesis *in vitro*. Most probably, the proteoglycans do not bind randomly along the fibril, but bind to specific binding sites, as indicated by the data in Fig. 2. This supposition is supported by the electron micrographs published by Scott & Orford (1981), showing that the dermatan sulphate proteoglycans are primarily localized at the *d*-band of the collagen fibrils, and by the fact that in different tissues there exists a proportionality between dermatan sulphate concentration and the surface area of collagen fibrils (Scott, 1984). Thus an adaptation of *d*-bands on different collagen fibrils caused by dermatan sulphate proteoglycan could explain the lateral alignment of the fibrils, with the light and dark bands being in register in the bundles of fibrils. It should, however, be noted that laterally aligned fibrils formed without proteoglycan can also show a pattern with light and dark bands in register (Birk & Lande, 1981).

Vogel *et al.* (1984) suggested that interactions between collagen and proteoglycans may be quite specific for both the type of proteoglycan and its tissue of origin. To study if any differences between human and rat collagen could be demonstrated, we compared the results obtained with human and rat type-I collagen. We found no differences in the rate of fibrillogenesis, the steady-state A_{400} , and the electron-microscopic appearance. Furthermore, the dermatan sulphate proteoglycan displayed the same extent of binding to both collagens, and affected fibrillogenesis and the electron-microscopic appearance in the same manner.

This work was supported by grants from the Danish Medical Research Council (Journal no. 12-4907), from Institut for eksperimentel klinisk forskning, University of Aarhus, from Aarhus Universitets Forskningsfond and from the Swedish Medical Research Council (7479).

REFERENCES

- Anderson, J. C., Labeledz, R. I. & Kewley, M. A. (1977) *Biochem. J.* **168**, 345–351
- Birk, D. E. & Lande, M. A. (1981) *Biochim. Biophys. Acta* **670**, 362–369
- Cöster, L. & Fransson, L.-Å. (1981) *Biochem. J.* **193**, 143–153
- Cöster, L., Fransson, L.-Å., Sheehan, J., Nieduszynski, I. A. & Phelps, C. F. (1981) *Biochem. J.* **197**, 483–490
- Danielsen, C. C. (1981) *Connect. Tissue Res.* **9**, 51–57

- Danielsen, C. C., Andreasen, T. T. & Mosekilde, L. (1986) *Calcif. Tissue Int.* **39**, 69–73
- Engvall, E. & Perlman, P. (1971) *Immunochemistry* **8**, 871–874
- Franzén, A. & Heinegård, D. (1984) *Biochem. J.* **224**, 59–66
- Gallagher, J. T., Gasiunas, N. & Schor, S. L. (1983) *Biochem. J.* **215**, 107–116
- Haigh, M. & Scott, J. E. (1986) *Fed. Eur. Connect. Tissue Soc. Meet.* 10th
- Heinegård, D., Björne-Persson, A., Cöster, L., Franzén, A., Gardell, S., Malmström, A., Paulsson, M., Sandfalk, R. & Vogel, K. (1985) *Biochem. J.* **230**, 181–194
- Jensen, J., Nørby, J. G. & Ottolenghi, P. (1984) *J. Physiol. (London)* **346**, 219–241
- Krusius, T. & Ruoslahti, E. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 7683–7687
- Neville, D. (1971) *J. Biol. Chem.* **246**, 6328–6334
- Öbrink, B. (1973) *Eur. J. Biochem.* **33**, 387–400
- Oegema, T. R., Laidlaw, J., Hascall, V. C. & Dziewiatkowski, D. D. (1975) *Arch. Biochem. Biophys.* **170**, 698–708
- Ottolenghi, P. & Jensen, J. (1983) *Biochim. Biophys. Acta* **727**, 89–100
- Reinsch, C. H. (1967) *Numer. Math.* **10**, 177–183
- Rosenthal, H. E. (1967) *Anal. Biochem.* **20**, 525–532
- Scott, J. E. (1980) *Biochem. J.* **187**, 887–891
- Scott, J. E. (1984) *Biochem. J.* **218**, 229–233
- Scott, J. E. & Orford, C. R. (1981) *Biochem. J.* **197**, 213–216
- Scott, P. G., Winterbottom, N., Dodd, C. M., Edwards, E. & Pearson, C. H. (1986) *Biochem. Biophys. Research Commun.* **138**, 1348–1354
- Stegeman, H. & Stalder, K. (1967) *Clin. Chim. Acta* **18**, 267–273
- Uldbjerg, N., Malmström, A., Ekman, G., Sheehan, J., Ulmsten, U. & Wingerup, L. (1983a) *Biochem. J.* **209**, 497–503
- Uldbjerg, N., Ekman, G., Malmström, A., Olsson, K. & Ulmsten, U. (1983b) *Am. J. Obstet. Gynecol.* **147**, 662–666
- Vogel, K. G. & Heinegård, D. (1985) *J. Biol. Chem.* **260**, 9298–9306
- Vogel, K. G. & Trotter, J. A. (1987) *Collagen Relat. Res.* **7**, 105–114
- Vogel, K. G., Paulsson, M. & Heinegård, D. (1984) *Biochem. J.* **223**, 587–597

Received 19 October 1987; accepted 10 December 1987