Type VI collagen is a major component of the human cornea

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Collagen type VI is shown to be present in the human cornea. This finding is based on comparative peptide mapping relative to type VI collagen isolated from placenta and on immunoblotting using antibodies specific for human type VI collagen. Scanning of polyacrylamide gels indicates that type VI collagen comprises as much as one quarter of the dry weight of the cornea. Indirect immunofluorescence shows this collagen to be distributed throughout the corneal stroma. Thus, type VI collagen must be considered a major component of the extracellular matrix of the human cornea.

Collagen *Extracellular matrix* *(Cornea, Human)*

1. INTRODUCTION

To date, of the 11 genetically different collagen types described [1], 4 have been detected in the human cornea [2,3]. These are type I, which is the predominant corneal component, type V and small amounts of types III and IV. Types I, III and V are co-distributed in the stroma, whereas type IV is located in Descemet's and Bowman's membrane.

Our attention has recently been focused on type VI collagen, a disuphide-linked protein consisting of a short triple helix bounded by relatively large globular domains [4–8]. Originally extracted from aorta and placenta, this collagen has since been detected in most other organs, including uterus, liver, kidney, skin, ligamentum and skeletal muscle [9,10].

Here, we demonstrate that type VI collagen forms a major portion of the extracellular matrix of the human cornea. This protein might therefore play an important role in the architecture of the corneal tissue.

This paper is dedicated to Professor C. Martius on the occasion of his 80th birthday

Abbreviations: SDS-PAGE, SDS-polyacrylamide gel electrophoresis; FITC, fluorescein isothiocyanate

2. EXPERIMENTAL

2.1 *Extractions*

Normal human corneas of individuals aged between 60 and 80 years were obtained at autopsy (Augenbank, Universitätsspital, Zürich). The frozen tissues were sliced individually with a microtome to give 50 μm sections, washed with distilled water and lyophilised. After measuring the tissue dry weight (6–7 mg), the slices were swollen in 2 ml of 0.5 M acetic acid. To each cornea so prepared, 2 mg pepsin (St. Louis, MO) was added and digestion performed at 4°C for 24 h. Undigested material was removed by centrifugation (30 000 × g, 10 min). The solubilised collagens were precipitated from the supernatant by adding NaCl to a final concentration of 2 M. The precipitate was redissolved, dialysed against 0.1 M acetic acid and lyophilised. The yield was determined gravimetrically.

The insoluble part of the cornea was washed

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with 0.5 M acetic acid, lyophilised and resuspended in 200 μl SDS sample buffer [11] containing 2% β-mercaptoethanol. Extraction was performed at 95°C for 10 min.

Finally, the solubilised collagens were analysed on 3–10% (w/v) SDS-PAGE [11]. Human type VI collagen isolated from placenta following established procedures [4–6] was used as a standard.

2.2 *CNBr cleavage*

Collagen samples were electrophoresed under reducing conditions on 8% polyacrylamide gels. After Coomassie blue staining, bands were cut out and subjected to CNBr cleavage in the gel slice [12]. Reaction was performed at 37°C for 3 h. The resulting peptides were separated on 10–15% (w/v) SDS–PAGE.

2.3. *Immunological procedures*

Antisera against type VI collagen from human fibroblasts were raised in rabbits and affinity-purified on type VI collagen from placenta [14,15]. The resulting antibodies recognised specifically the pepsinised α_1(VI)- and α_2(VI)-chains, as has been found for similar preparations of antibodies [15]. Western blotting was carried out following a modification of the procedure of Towbin et al. [13], in which the second antibody was replaced by [^{125}I]iodinated protein A [8]. Bands were visualised by autoradiography.

Indirect immunofluorescence using FITC-labelled goat anti-rabbit second antibodies (Miles, Naperville, IL) was performed on frozen sections (4 μm) of human cornea as described in [16].

3. RESULTS AND DISCUSSION

Pepsin digestion solubilised 5–10% of the original dry weight of the cornea. A subsequent extraction with SDS sample buffer yielded a further 5–15% depending on the individual cornea. This variability was found to be largely due to decreased extractability with increasing age of the cornea.

Analysis of the extracted collagens on SDS-PAGE showed under non-reducing conditions the typical pattern of type I collagen (fig.1). Very little type V collagen could be detected. In addition, a blurred band migrating with an apparent molecular mass of 350 kDa was observed. Upon

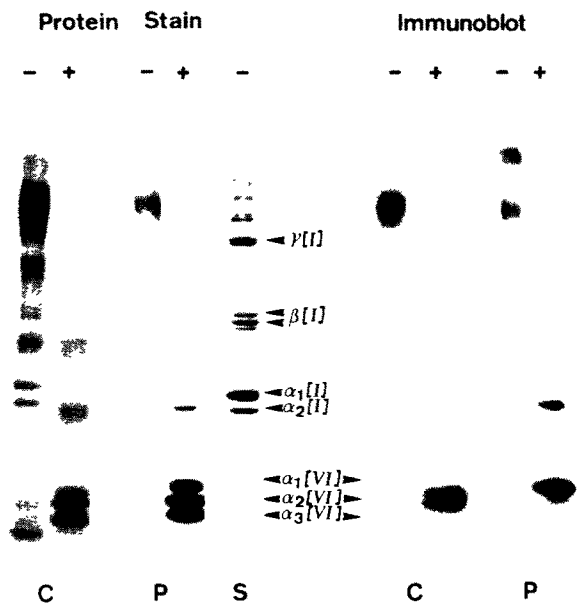

Fig.1. Comparison of pepsin-extracted collagens from human cornea (C) with type VI collagen from human palcenta (P). Proteins were separated on 3–10% SDS-PAGE under reducing (+) or non-reducing (−) conditions and either stained with Coomassie blue or processed for immunoblotting. Human type I collagen was used as standard (S).

reduction this band disappeared and 3 distinct components with relative molecular masses of 53, 49 and 44 kDa were generated. Bands of lesser intensity also appeared at about 90 and 150 kDa, possibly corresponding to dimeric and trimeric forms of the faster migrating components. An almost identical pattern was obtained after electrophoresis of a type VI collagen standard from placenta. Only a slight difference in the mobilities of the placental α_1(VI)-chain and the corresponding corneal material was noted.

Immunoblotting experiments using affinity-purified antibodies specific for the α_1(VI)- and α_2(VI)-chains confirmed the presence of type VI collagen in human cornea. Unreduced as well as reduced samples showed a positive immunoreaction (fig.1). In addition to the bands in the α-region of type VI, the proposed dimeric and trimeric forms were also recognised by the antibodies.

Peptide mapping provided the final proof for the occurrence of type VI collagen in human cor-

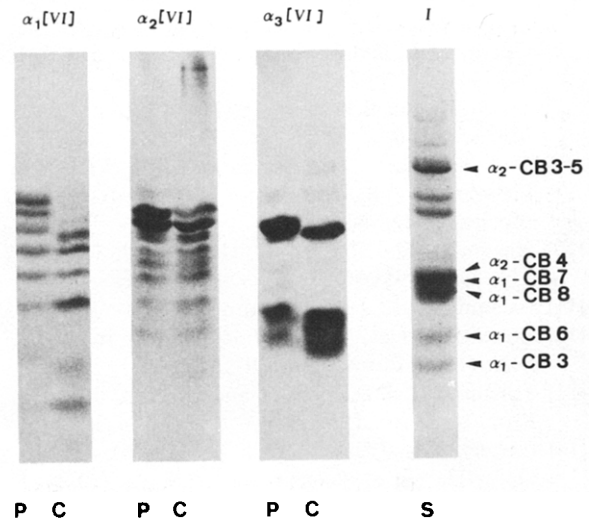

Fig.2. Analyis of CNBr fragments of the individual type VI collagen chains from human palcenta (P) and human cornea (C) on 8–15% SDS-PAGE. CNBr fragments from bovine type I collagen were used as standard (S).

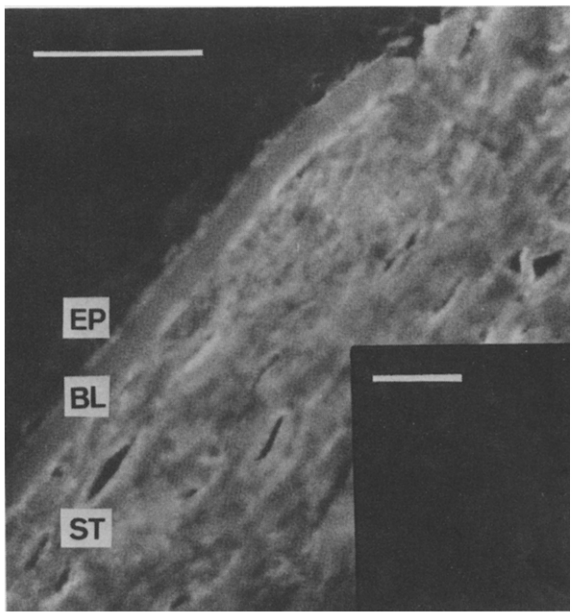

Fig.3. Indirect immunofluorescence picture of the epithelial region of the cornea (bar: 100 μm). EP, epithelium; BL, Bowman's layer; ST, stroma. Inset: control.

neas (fig.2). CNBr fragments of the individual type VI chains derived from placental and corneal tissues showed essentially the same mobilities on SDS-PAGE. In general, the placental samples contained a higher percentage of uncleaved or only partially cleaved material – possibly as a result of more extensive oxidation of methionine residues. The slightly different mobilities of two peptides derived from the α_1-chains is consistent with the different mobilities of the uncleaved α_1 (VI)-chains noted above. This observation may be explained by differences in the pepsin cleavage and/or post-translational modifications.

Indirect immunofluorescence analysis demonstrated type VI collagen to be distributed throughout the corneal stroma (fig.3). Weak staining was observed in the Bowman's layer, whereas epithelium and endothelium did not react with the antibodies. Consistent with our findings, two recent reports mentioned the occurrence of type VI collagen in mouse cornea based on immunofluorescence [9,17]. These studies also described immunoreactivity of the stroma, but not of the adjacent basement membranes. In placenta and aorta, collagen type VI appeared to be located between the cross-striated fibrils of the interstitial collagens [9]. How type VI collagen is integrated into the tight organisation of the corneal extracellular matrix is not yet known and must await further investigations.

To determine the proportion of collagen type VI in cornea, we scanned the Coomassie blue-stained gels. In both pepsin and SDS extracts, type VI collagen was the predominant component, comprising 50–70% of the protein applied to the gels. In relation to the original dry weight of the cornea, 7–10% collagen type VI could be extracted. However, this percentage includes only the pepsin-resistant domain, which has a molecular mass of approx. 150 kDa. The intact type VI molecule with its large globular domains has a molecular mass of about 400 kDa. This consideration leads to the conclusion that the intact type VI collagen must comprise as much as one quarter of the corneal dry weight. Type VI collagen therefore has to be considered a major component of the human cornea and might play an important role in the organisation of its extracellular matrix.

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