An insoluble structural glycoprotein a major constituent of the zonula Zinni

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Experiments were carried out to determine the localization of the insoluble structural glycoprotein which remains after exhaustive degradation of the bovine and rabbit lens capsules with high-purity collagenase. The material can be extracted from the residue with 0.1 M β-mercaptoethanol at room temperature. Most of this material was shown to be present in the capsule of the equatorial region of the lens. Furthermore, preparations of lens capsule containing the extracapsular fibrils of zonula Zinni were shown to contain about as much of this structural glycoprotein as the whole capsule itself. The glycoprotein appears to be a major constituent of the zonula fibrils and the composition of its carbohydrate suggests a close relationship to the glycoprotein of the vitreous body previously obtained in our laboratory as an insoluble precipitate after degradation of the hyaluronic acid of the vitreous.*

Preparations of bovine and rabbit lens capsules obtained in such a way that the capsule is separated from the zonula Zinni at the border line of the equatorial region of the lens have been shown to contain a glycoprotein which remains as a residue after exhaustive digestion of the lens capsule by high-purity collagenase.[1, 2] This glycoprotein has been referred to as insoluble structural glycoprotein (ISGP). It has been, furthermore, shown that this glycoprotein can be extracted to a large extent by treatment of the capsule with 5 M guanidine hydrochloride at pH 4 and completely by guanidine hydrochloride 5 M at pH 7 in combination with 0.1 M β-mercaptoethanol. This treatment extracted only a small part of the glycoprotein linked to collagen of the capsule.[3] It has been clear that the ISGP is either bound by weak noncovalent bonds to the collagen or is a constituent of a particular structural part of the capsule. Differences in the texture of the collagen of the lens capsule have been reported by our laboratory already in 1954[4] insofar as the equatorial region seemed to contain less collagen per unit weight and differ also in the content of the disaccharide linked to collagen. One possibility, therefore, to be considered was that ISGP was present only in a particular region of the lens capsule. The other possibility was that it was a constituent of the capsular zonula Zinni which obviously was removed together with the lens capsule in our procedure. To test these two possibilities, we carried out a series of experiments described below.

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This work was supported by Grants CY 00348 of the National Eye Institute and CA 02075 of the National Cancer Institute.

Submitted for publication July 29, 1974.

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*The term extracapsular zonula is used to designate zonula fibrils which do not reach the area of the lens. The term capsular zonula designates zonula fibrils within the area of the lens.

Table I. Topical distribution of the hexosamine of the structural glycoprotein in the bovine lens capsule

Experiment	*μg Ham°* / *capsule*	*μg Ham°* / *mg. OHPr*
1.		
a. Anterior polar region	0	
b. Anterior equatorial region	1.9	8.1
Posterior total capsule	1.16	7.4
2.		
a. Total equatorial region	3.3	2.6
b. Posterior polar region	0.07	0.01

Ham = hexosamine; OHPr = hydroxyproline.
°All values averages from 100 to 200 capsules.

Experimental

Experiments were carried out on the lens capsules from about 30 month-old steers and about three month-old calves. The eyes were brought packed in ice from the slaughter house and the lens capsules immediately prepared in two different ways. According to one procedure, the zonula Zinni, as in previously reported experiments, was cut with a scissor at the border line of the equatorial region of the lens. The lens was taken out and the capsule was then cut at the border line of the equatorial region, the anterior and posterior regions stripped off separately, and the epithelium of the anterior capsule was stripped off with a scalpel. To compare the composition of the polar region of the anterior and posterior capsule with the equatorial region, the central part of the two parts of the capsule was either stamped out with a troquar, the diameter of which corresponded to ⅔ of the diameter of the anterior capsule, or the equatorial region was separated from the polar region by cutting the capsule still connected with the lens along a circular line at the anterior and posterior surface of the lens and separating the border part with an approximate diameter of ⅓ of the total diameter of the anterior and posterior capsule, respectively. In a second type of experiment where the extracapsular zonula Zinni fibrils were to be investigated, the zonula was laid bare as far toward the ciliary body as feasible and most of the pigment pushed aside with a fine scissor. The zonular fibrils were then cut peripherally as far away from the border of the lens as possible and the equatorial region of the capsule was separated according to the second procedure for the separation of this region described above from the polar, anterior, and posterior part of the capsule.

Analytic procedures. The ISGP was prepared according to the previously described procedure[1] by exhaustive digestion with high-purity collagenase (Worthington) and extraction of the insoluble residue with 0.1 M β-mercaptoethanol for 16 hours at room temperature, pH 7.4. The mercaptoethanol extract was exhaustively dialyzed first in running and then in distilled water. Hydroxyproline was determined by the Neuman-Logan method,[5] hexosamine by the indole HCl reaction,[6] fucose and hexose by modifications of the cysteine H_2SO_4 reaction,[7, 8] and hexuronic acids by the carbazole reaction.[9]

Results

Comparison of the content in ISGP in the equatorial and polar regions of the lens capsule. The hexosamine in ISGP was separately determined in the polar region of the anterior and posterior capsule as well as in the equatorial region of the anterior and the whole capsule. The content in hexosamine in micrograms per capsule and per milligram of hydroxyproline are listed in Table I. As can be seen in the polar regions of the anterior and posterior capsule which represented about ⅔ of the surface area of the total lens capsule, only very small amounts of hexosamine were found in the remaining residue after exhaustive collagenase treatment.

The main bulk (over 90 per cent) of the total ISGP is found in the two equatorial regions. These findings could be interpreted either that the level of ISGP is related to differences in the structure of the collagen matrix of the equatorial and polar regions or that it is a constituent of the capsular zonula Zinni which can be assumed to be present in the equatorial region of the anterior as well as the posterior lens capsule. In a second series of experiments, therefore, we compared in two preparations of the equatorial region of capsules from lenses separated from the extracapsular zonula Zinni at the borderline of the capsules with that in the equatorial regions of the capsules which were separated as close to the ciliary body as possible and contained extracapsular zonula Zinni fibrils.

The results of these determinations are presented in Table II. As can be seen, the hexosamine content per preparation appears about twice as high in the preparation containing the total zonula Zinni as in

Table II. Content and molar ratios of sugar constituents of the insoluble structural glycoprotein of the lens capsule of steer and calf obtained with and without the extra capsular fibrils of the zonula Zinni

Experiment No.	*Material*	*μg Ham* / preparation*	*μg Ham* / mg. OHPr*	*F / Ham*	*F / H*	*Wet weight of extracapsular zonula Zinni fibrils (mg./preparation)*	*Wet weight of capsule/mg. preparation*
1.	Steer						
	a. capsule	4.75	11.	0.26	0.11		
	b. capsule +zonula fibrils	10.0	25.6	0.27	0.16	22	88
2.	Steer						
	a. capsule	4.50	9.65	0.35	0.19		
	b. capsule +zonula fibrils	8.8	17.4	0.30	0.20	31	107
3.	Calf						
	a. capsule	3.5		0.38	0.12		
	b. capsule +zonula fibrils	7.8		0.30	0.16	24.6	69
4.	Calf						
	a. capsule	3.4	21.8	0.25	0.11		
	b. capsule +zonula fibrils	5.5	35.8	0.25	0.13	16	67

All values in milligrams per 100 capsules and per milligram of hydroxyproline.
H = hexose; F = Fucose; Ham = hexosamine; OHPr = hydroxyproline.
*Determined as average from 26 to 50 capsules.

those preparations cut at the borderline of the lens. The ratio of hexosamine to hydroxyproline is, in the first preparations, about twice as high which can be expected if the extracapsular zonula fibrils themselves do not contain any significant amount of collagen.

As the extracapsular zonula fibrils were slightly contaminated with the pigment from the ciliary processes and possibly the latter could contain an insoluble structural glycoprotein, experiments were carried out to determine the possible effect of this contamination on the content in hexosamine of the whole preparation. To this end, an amount of pigment was collected about twice the weight of the extracapsular zonula fibrils themselves as calculated from the difference in weight between the two different preparations of the capsule. The pigment was treated exactly in the same way as the capsules by exhaustive digestion with collagenase and extraction of the remaining residue with β-mercaptoethanol. A residue was obtained which contained hexose, fucose, and hexosamine, but their amount per wet weight of the pigmented material was about the same as the differences in the ISGP content between the preparation of capsules containing the extracapsular zonula fibrils and those without them. As only minimal amounts of pigment were attached to the zonula fibrils, the differences between the two different preparations of the capsules can only be interpreted as due to the presence of the ISGP in the extracapsular zonula fibers.

Discussion

The results of this series of experiments seem to show clearly that the extracapsular fibrils of the zonula Zinni contain a high concentration of the ISGP which can, to a large extent, be extracted with 0.1 M β-mercaptoethanol in 16 hours at RT at pH 7.4. To extract ISGP completely, it seems necessary to preincubate the capsule with collagenase for a short time. The capsular ISGP then becomes accessible to extractions with β-mercaptoethanol. It would appear that only a small part of the collagen must be removed for successful ex-

traction. Moreover, the ISGP is present in the fibrillar extracapsular zonula Zinni in about the same amount as in the equatorial region. These observations strongly suggest that the fraction of ISGP in the equatorial region is associated with the capsular part of the zonula Zinni.*

Our results on the ISGP in the extracapsular zonula Zinni permits an estimation of how much of the dry weight of this part of the zonula is represented by ISGP. In the experiments on the steer capsule the maximum weight of the extracapsular zonula can be calculated as the difference between the weight of the equatorial region of the capsule itself and the combined weight of this equatorial region plus the extracapsular zonula Zinni. This difference varies in the two samples, each of 100 steer capsules, between 0.8 and 1.10 grams of wet weight. The ISGP in this extracapsular part of the zonula can be calculated as the difference of the β-mercaptoethanol extractable material in the preparation with and without the extracapsular zonula. This amounts to about 4.6 μg of hexosamine per preparation. If we assume the same ratio between hexosamine and hexose and hexosamine and fucose as was previously found in ISGP of the bovine capsule,[1] then we can calculate the total carbohydrate in the extracapsular zonula to be about 18 μg of carbohydrate per preparation. If we assume the ratio of 1:10 between carbohydrate and protein of ISGP as previously found for the ISGP of the equatorial region of the capsule,[1] we calculate that the total glycoprotein per extracapsular zonula is about 200 μg. Thus, there is 200 μg per 22 to 31 mg. of wet weight of extracapsular zonula per preparation. If we assume that the per cent dry weight of the zonula Zinni is the same as that of the lens capsule, then about 200 μg of ISGP would correspond to about 3 to 4.5 mg. of the dry weight. Although the ISGP can be considered to be a major component of the zonula, it does not appear to be responsible for its morphologic integrity. The pre-extraction of the capsule and extracapsular zonula with chymotrypsin in a concentration of the enzyme as high as that used in cataract surgery does not release any ISGP from the zonula fibrils, nor could we find any degradation of the isolated ISGP by chymotrypsin after 90 minutes digestion at RT in a solution containing 750 units per 10 ml.

The composition of the carbohydrate of the ISGP appears of particular interest because of its similarity with the composition of the carbohydrate of a glycoprotein prepared in our laboratory in 1958[10] from the bovine vitreous body. When the collagen fibrils in the vitreous were removed by centrifugation and the supernatant dialyzed exhaustively, the latter remained clear. If, however, this supernatant was treated before dialysis with hyaluronidase, a precipitate appeared in the dialysant which proved to be glycoprotein material containing about 10 per cent carbohydrate. This carbohydrate consisted of three hexoses, mannose, galactose, and glucose in about the same proportion as the carbohydrate in ISGP. It also contained fucose with a ratio of fucose to total hexose of about 0.14 as compared to the ratio in ISGP of 0.11 to 0.15. The ratio of fucose to hexosamine in the vitreous material was about ⅓ lower than in ISGP. It should be noted, however, that it has not been determined whether the material from the vitreous was free of hexuronic acid. This might account for the excess hexosamine in this material as compared to hexosamine in ISGP. Further experiments in this direction are therefore required to establish the close compositional or structural relationship between the two glycoproteins. The similarity in the composition of the carbohydrate of the two glycoprotein preparations appears of particular interest in view of the probable origin of the zonula Zinni fibrils from vitreous constituents. In view of our results, the hypothesis appears reasonable that one part of the material of the zonula fibrils is derived from the glycoprotein of the vitre-

*It would appear that there is collagen associated with the capsular zonula Zinni.

ous. This transformation to an insoluble glycoprotein material could be achieved by the degradation of the hexuronic acid.

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