A C T A B I O C H I M I C A P O L O N I C A

Vol. 36 1989 No. 1

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SOLUBLE NON-COLLAGEN PROTEINS OF BOVINE VITREOUS HUMOUR

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Received 17 May, 1988

Bovine vitreous humour contains 0.6 ± 0.04 mg of proteins per cm^3. Three main protein fractions were obtained by CM-cellulose chromatography and their homogeneity was confirmed by isoelectric focusing and SDS-gel electrophoresis. The M_r and pI values of the separated fractions were: fraction I, 67000 and 4.02; fraction II, 59000 and 5.22; fraction III, 43000 and 7.48, respectively.

It is well known that the vitreous humour contains both collagen and non-collagen proteins. The vitreous humour collagen has recently been very well characterized [1 - 4]. Bovine vitreous humour contains about 0.4 - 0.8 mg of soluble proteins per cm^3. It is suggested that some of them originate from the plasma [5]. Since this group of proteins has not been characterized so far we decided to make some studies on their physical and chemical properties.

MATERIAL AND METHODS

The studies were made on fresh vitreous humour taken from bovine eye balls obtained from the local slaughter-house. The samples investigated did not contain pathological components such as blood or pus. The material taken from 20 - 40 eyes was pooled and centrifuged for 1 h, 20000 × *g*, at 4°C.

Protein concentrations in the vitreous humour of 20 bovine eye balls was determined by the biuret method. Hydroxyproline was estimated according to Bergman & Loxley [6].

Molecular sieve chromatography was performed on Sephadex G-100. The column (1.5 × 90 cm) was equilibrated with 0.05 M Tris/HCl + 1 M NaCl, pH 7.4. The samples of 3 - 4 cm^3 were applied to the column. The proteins

were eluted from the column with 0.05 M Tris/HCl + 1 M NaCl, pH 7.4. Fractions of 5 cm^3 were collected and their absorbance at 280 nm was measured.

CM-cellulose chromatography. The samples were dialysed against a 0.02 M acetate buffer + 1 M urea, pH 4.8 (starting buffer) and applied to a CM-cellulose column (1.8 × 20 cm) equilibrated with the same buffer. The proteins were eluted with the starting buffer and then with a linear NaCl gradient (0.0 - 0.4 M) in the same buffer. Fractions of 5 cm^3 were collected and their absorbance at 280 nm was measured.

DEAE cellulose-chromatography. The samples were dialysed against 0.02 M Tris/HCl, pH 8.0 (starting buffer) and applied to a DEAE-cellulose column (1.8 × 20 cm) equilibrated with the same buffer. The proteins were eluted with the starting buffer and then with a NaCl gradient (0.0 - 0.5 M) in the same buffer. Fractions of 5 cm^3 were collected and their absorbance at 280 nm was measured.

Isoelectric focusing. The pH gradient of 3.5 - 10.0 was obtained in 110 cm^3 isoelectric-focusing column (LKB) with the use of Ampholine in a sucrose gradient according to Vesterberg & Svensson [7]. The focusing was carried out for 48 h at a constant voltage of 500 V and temperature of 8°C. Fractions of 2 cm^3 were collected and the pH measurements of each of them were made with a pH-meter, type N-517 (Mera-Elwro, Poland) standardized with buffers of pH 4.0 and 8.0. All the fractions were dialysed against distilled water for 3 days in cold room and the protein concentration was measured by the microbiuret method [8].

SDS-polyacrylamide gel electrophoresis. The proteins were incubated at 37°C for 2 h in 0.01 M phosphate buffer + 1% SDS + 1% β-mercaptoethanol, subjected to electrophoresis on 5% SDS-polyacrylamide gel according to the method of Weber & Osborn [9] and M_r values for various proteins were estimated using following substances as standard M_r markers (Pharmacia, Uppsala): lactalbumin - M_r 14 000, soybean trypsin inhibitor - M_r 20 000, carbonic anhydrase - M_r 30 000, ovalbumin - M_r 43 000, bovine serum albumin - M_r 67 000, phosphorylase "*b*" - M_r 94 000.

Amino acid analysis. The proteins were hydrolysed in 6 M HCl at 110°C for 24 h under nitrogen and then analysed for amino acid composition on amino acid autoanalyser, type AAA 881, Microtechna (Czechoslovakia).

RESULTS AND DISCUSSION

It was found that the total content of protein in fresh bovine vitreous humour is 1.7 ± 0.5 mg per cm^3. Most of them, including collagen (hydroxyproline containing material) are sedimented during centrifugation. The soluble fraction of bovine vitreous humour contains 0.6 ± 0.04 mg of proteins per cm^3. No collagen was found in this material. During gel filtration

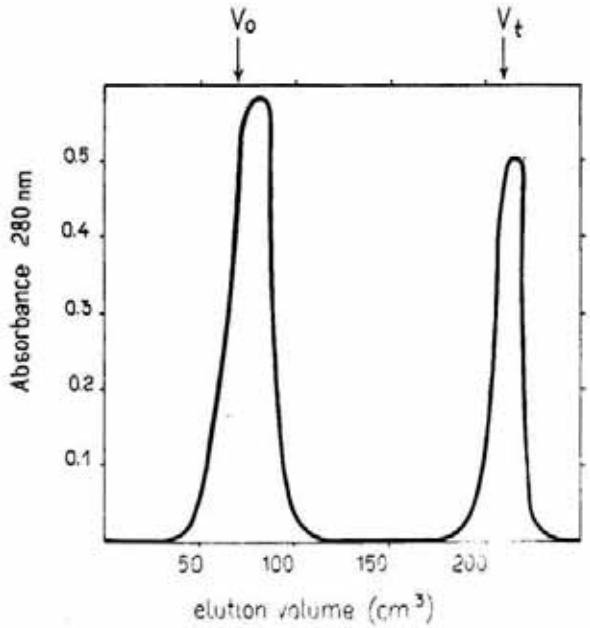

Fig. 1. Molecular sieve chromatography of soluble vitreous proteins on Sephadex G-100. (——), Absorbance at 280 nm

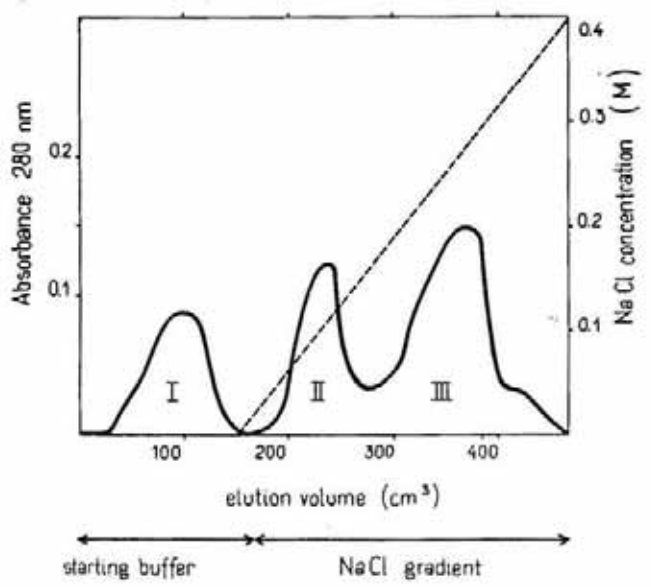

Fig. 2. CM-cellulose chromatography of soluble proteins of vitreous humour. (——), Absorbance at 280 nm; (- -), NaCl concn. (M)

on Sephadex G-100, the total amount of soluble vitreous proteins was eluted as a symmetric peak located close to the void volume of the column. Another high peak was found near the total volume of the column (Fig. 1). It contained low molecular, dialysable, nonprotein material, probably free amino acids and small peptides.

Since it proved impossible to fractionate vitreous humour proteins by molecular sieve chromatography we tried to separate them by ion-exchange chromatography. As it is shown in Fig. 2 vitreous humour proteins are separated by CM-cellulose chromatography into 3 fractions. Fraction I

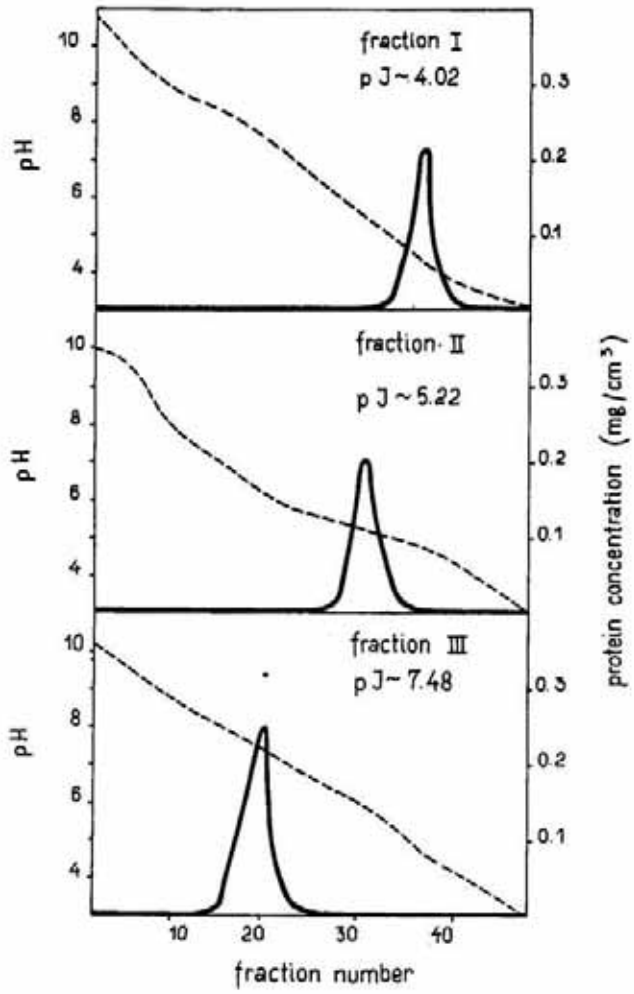

Fig. 3. Isoelectric-focusing of protein fractions obtained by CM-cellulose chromatography. (——), Protein concn. (mg/cm³); (- -), pH

was not bound by CM-cellulose and was eluted with the starting buffer. Fraction II was eluted with 0.1 M NaCl and fraction III with 0.27 M NaCl.

Further rechromatography of fraction I on DEAE-cellulose column did not result in any fractionation of protein material. The total amount of it was eluted as a single symmetric peak (not shown).

Fractions I, II and III were concentrated by vacuum dialysis and subjected to isoelectric focusing. The results are presented in Fig. 3. Linear pH gradients were obtained. Each fraction (I, II, III) focused as a single symmetric peak with isoelectric point of 4.02, 5.22 and 7.48, respectively. To better characterize the soluble vitreous humour proteins we subjected them to SDS-polyacrylamide gel electrophoresis (Plate 1). Three separate bands a, b and c were obtained. In additional experiments it was found that the bands a, b, c correspond to fractions III, II and I, respectively. Their molecular weights were: fraction I 67 000, fraction II 59 000 and fraction III 43 000.

The amino acid composition of bovine vitreous humour proteins (fractions I, II, III) is shown in Table 1. Fraction I contains a large amount of aromatic (Tyr, Phe) and branched (Leu, Val) amino acids. They constitute about 30% of the total amino acid residues. The ratio of acidic to basic amino acids is 1:0.97, which confirms the anionic character of this protein.

Table 1

Amino acid composition of bovine vitreous humour soluble proteins

Amino acid	Fraction I	Fraction II	Fraction III
	Residues per 1000 residues		
Lysine	91	82	96
Histidine	34	29	35
Arginine	41	40	43
Aspartic acid	81	104	87
Threonine	71	74	61
Serine	74	96	104
Glutamic acid	89	82	78
Proline	41	61	17
Glycine	74	77	133
Alanine	95	77	78
Cysteine	0	0	0
Valine	68	66	87
Methionine	14	3	9
Isoleucine	30	50	43
Leucine	101	102	87
Tyrosine	34	21	17
Phenylalanine	64	45	43
Tryptophan	not determined		

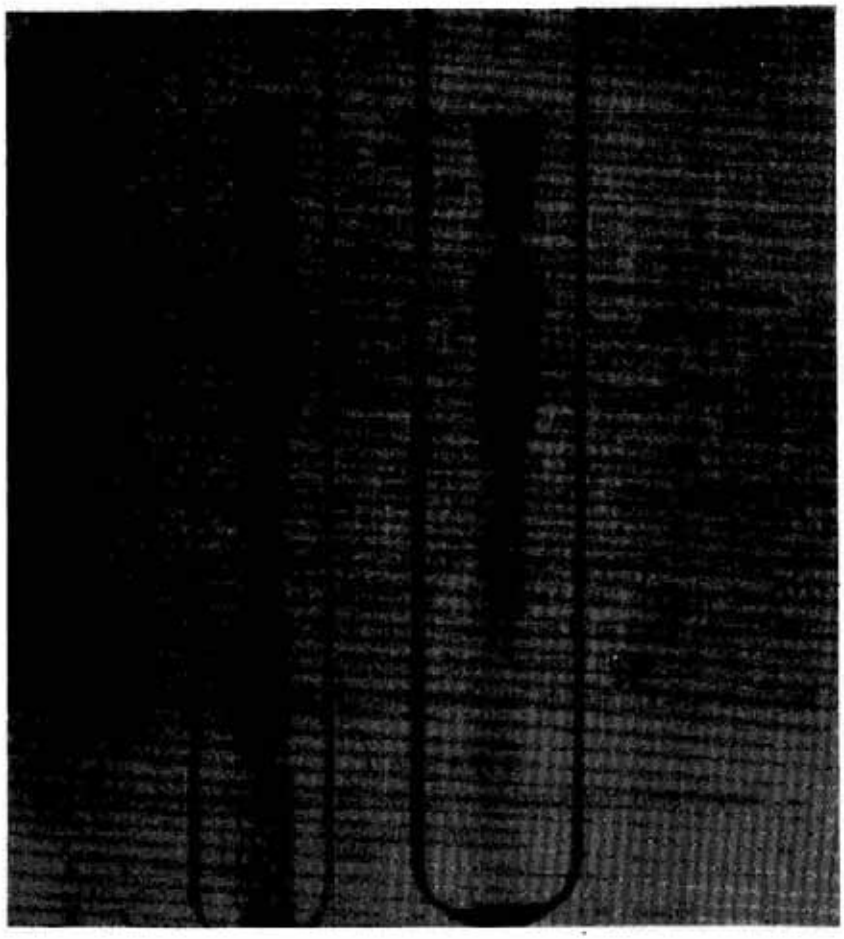

Plate 1. SDS-polyacrylamide gel electrophoresis of soluble vitreous humour proteins in comparison with standards of kown molecular weight

Fraction II has an amino acid composition similar to that of fraction I, but it contains smaller amounts of aromatic amino acids. The ratio of acidic to basic amino acids is 1:0.81. It is worth noting that the number of methionyl residues in this fraction is five times lower than in fraction I.

Fraction III has a quite different composition as compared with the other fractions. It contains much more glycine and smaller amounts of aromatic amino acids (especially Tyr). The ratio of acidic to basic amino acids is 1:1.05. This explains the slightly cathionic character of this fraction.

None of the proteins investigated contains cysteine, although hydrolysis was performed under conditions which should protect this amino acid from decomposition.

It may be concluded from our studies that bovine vitreous humour contains 3 non-collagen protein fractions. The physiological role of these proteins is not known. It may be concluded from our immunological studies [10] that at least some of them originate from plasma.

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