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Journal of Chromatography B, 751 (2001) 349–355

JOURNAL OF CHROMATOGRAPHY B

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Chromatographic identification of a biochemical alteration in the aqueous humour of megalophthalmic Black Moor goldfish

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Received 30 May 2000; received in revised form 1 September 2000; accepted 1 September 2000

Abstract

Purpose: To observe if any biochemical abnormalities exist between the eye of megalophthalmic and non-megalophthalmic goldfish by high-performance liquid chromatography (HPLC). *Method*: Aqueous humour and sera from megalophthalmic and non-megalophthalmic goldfish were subjected to HPLC and monitored by photodiode array detection (Waters, MA, USA). *Results*: An unusual accumulation of a compound with a UV absorption maximum at 290 nm was observed in the aqueous humour of megalophthalmic eye. This compound was also present in the sera of both normal goldfish and one of its megalophthalmic mutant. However, it was significantly elevated in the aqueous humour of the megalophthalmic eye only. This compound concentration was very high in the eye of small fish and its concentration increased only slightly with the expansion of the eye in larger fish. *Conclusions*: The presence of this compound in the serum and aqueous humour indicates a specific systemic metabolic variation in Black Moor goldfish not seen other animal species we had studied (humans, bovine, chick, rabbits and rats). The marked elevation of this compound in the megalophthalmic eye indicates a possible association of this compound with the metabolic variation accounting for the expansion of the eye in megalophthalmic goldfish.

Keywords: Goldfish; Megalophthalmia; Aqueous humour

1. Introduction

One of the well-established biological features of myopia is the elongation of the affected eyeball. The biochemical events leading to scleral expansion are not known. The Black Moor goldfish is a mutant strain of the common goldfish, *Carassius auratus*. It is characterised by the profound enlargement and protrusion of its eye, as well as by its all black colouring. The eye in this strain of goldfish is strongly myopic [1,2]. Although the aqueous humour of normal animals appears to be unremarkable in terms of the presence of unique substances, certain substances do accumulate in it after the eye has been experimentally stimulated. For instance, the post ischemic increase of cAMP [3] and PGE2 [4], the increase of endothelin-1 after laser trabeculoplasty [5] and the appearance of cholesterol crystals subsequent to retinal detachment [6]. This study is directed at examining the aqueous humour of normal

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0378-4347/01/$ – see front matter
PII: S0378-4347(00)00493-X

goldfish (*C. auratus*) and its megalophthalmic mutant, the Black Moor goldfish, for evidence of specific biochemical alteration unique to the megalophthalmic eye.

2. Experimental

2.1. Animal specimens

All goldfish were obtained from a single commercial vendor in Hong Kong. The fish were deeply anaesthetised by immersing them in 0.05% MS222 (Sigma, St. Louis, MO, USA). Aqueous humour was obtained by direct needle puncture through the cornea. The body was wiped dry and whole blood was obtained via cardiac puncture with a 25G needle. It was kept on ice for 30 min and the sera were separated from the blood cells by centrifugation at 960 *g* for 30 min.

The normal and megalophthalmic goldfish were divided into three groups based on their body length as measured from snout to base of tail (Table 1). Archimedes' principle was used as the basis for measuring the size of the eye: the eye volume was determined according to the volume of water it displaced. There was little variation in the eye volume of the three groups of normal goldfish. Amongst the megalophthalmic goldfish, the eye volume increased as the fish grew larger.

2.2. Analysis of aqueous humour and sera by high-performance liquid chromatography (HPLC)

The chromatograms of normal and megalophthalmic goldfish samples were compared using a HPLC system equipped with a photodiode array detector (Waters, Milford, MA, USA) and a carbohydrate column (256×4.5 mm, Waters). The mobile phase consisted of 20 m*M* ammonium phosphate. A 1-l volume of aqueous humour was injected into the column and eluted at 1 ml/min. Serum specimens were first diluted fivefold with 20 m*M* ammonium phosphate and then centrifuged to remove any insoluble proteins. A 5-μl aliquot was then injected into the column.

2.3. Analysis for albumin concentration by HPLC

Albumin concentration in the aqueous humour was analysed by injecting 1 μl of aqueous humour into a Protein-Pak 125 column (Waters) using 0.1 *M* sodium chloride as the mobile phase at a flow-rate of 1 ml/min. The eluent was recorded at 280 nm. The concentration of albumin in the sample was calculated from the peak height using bovine serum albumin as a standard.

3. Results

3.1. Marked elevation of an unknown compound with a UV absorption maximum at 290 nm in the aqueous humour of megalophthalmic goldfish eye

The 215 nm chromatogram indicated the presence of a group of peaks with short retention times of less than 4 min. There was no obvious difference between the normal and megalophthalmic eye (com-

Table 1
Sizes of the goldfish specimens

Fish size		*n*	Normal goldfish	*n*	Black Moor goldfish
Small	Body length (cm)	6	4.5±0.2	11	4.9±0.1
	Eye volume (ml)		0.2±0.0		0.4±0.0
Medium	Body length (cm)	7	5.1±0.1	7	6.3±0.3
	Eye volume (ml)		0.2±0.0		1.3±0.1
Large	Body length (cm)	6	8.3±0.2	6	9.4±0.2
	Eye volume (ml)		0.5±0.0		3.2±0.4

pare the lower tracings of Fig. 1a and b). Aromatic compounds in the aqueous humour were detected by light absorption at 290 nm. A dominant peak was observed in the aqueous humour of a megalophthalmic eye (Fig. 1a, upper tracing). Only a trace amount of this compound was observed in the normal eye (Fig. 1b, upper tracing). The absorption spectrum of this compound (Fig. 2b, peak No. 6) is very different from all the other peaks as shown in Fig. 2a and b.

The amount of this unknown compound in the megalophthalmic eye was similar amongst all three sizes of goldfish (Table 2). Judging from the quantitative difference between the two strains of goldfish and the appearance of large quantities of this compound in the small megalophthalmic fish, the elevation of this compound must have occurred at the beginning of eye expansion.

3.2. Occurrence of the compound with absorption maximum at 290 nm in fish serum

The unknown compound observed in goldfish aqueous humour was also detected in the sera of both the normal and megalophthalmic goldfishes (Table 3). The identity of the major peaks in the aqueous humour was identical to that observed in the serum based on retention time (Fig. 3a) and UV absorption spectrum (Fig. 3b). Due to a large standard deviation, the difference between the sera of normal and

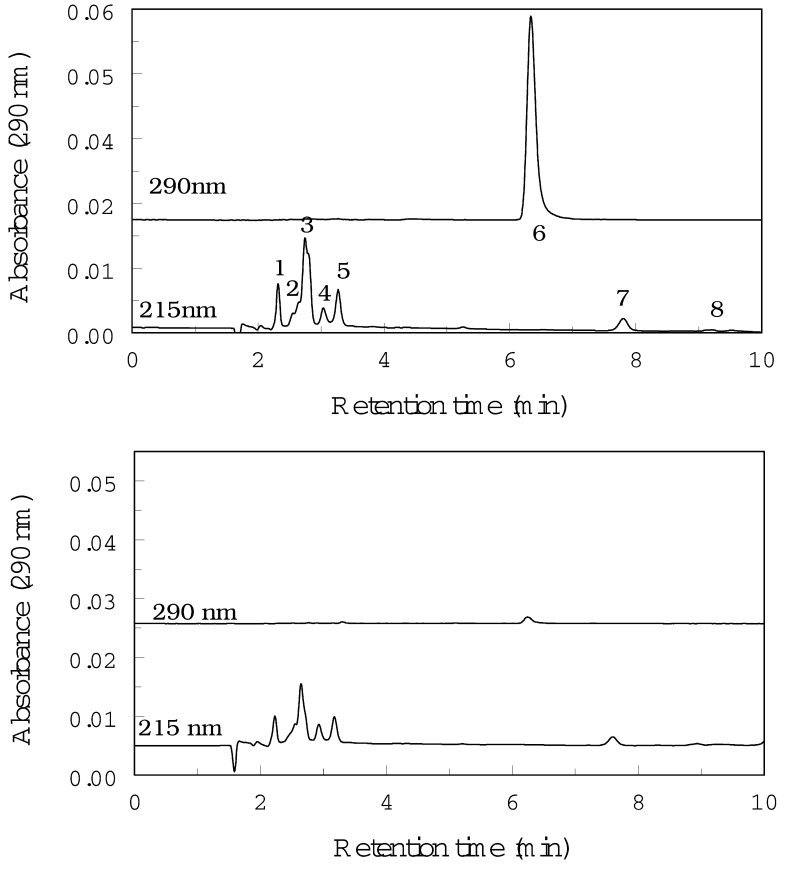

Fig. 1. (a) UV absorption chromatogram of the aqueous humour obtained from a megalophthalmic eye of a small size goldfish. Upper tracing – 290 nm chromatogram. Lower tracing – 210 chromatogram. (b) UV absorption chromatogram of the aqueous humour obtained from a normal eye of a small size goldfish. Upper tracing – 290 nm chromatogram. Lower tracing – 210 chromatogram.

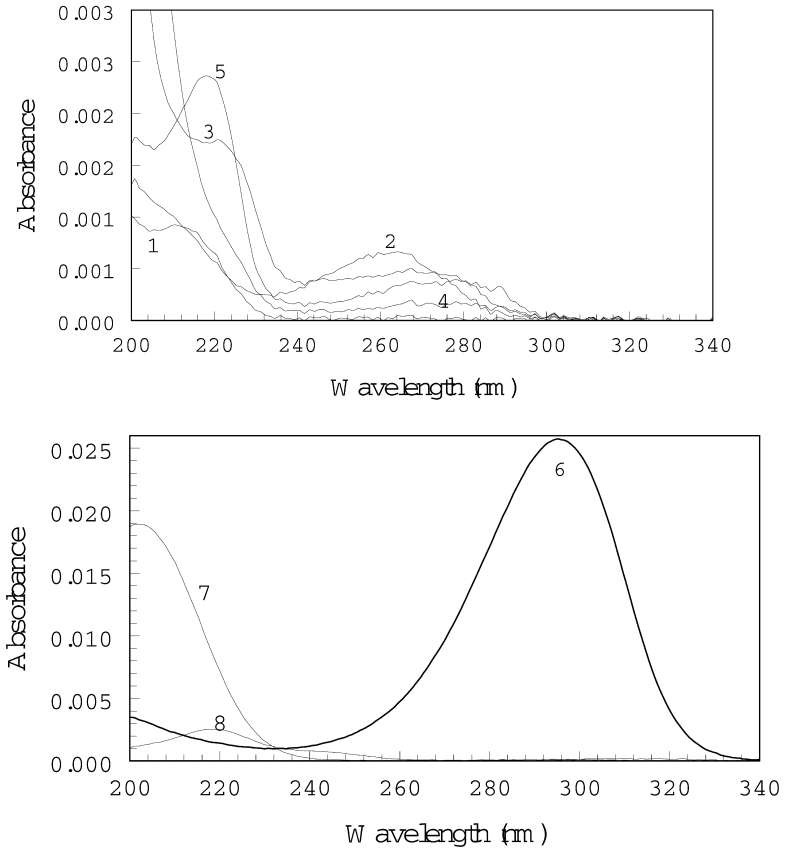

Fig. 2. (a) Absorption spectrum of peaks 1–5 of Fig. 1a. (b) Absorption spectrum of peaks 6–8 of Fig. 1a.

Table 2
Peak height (absorbance 290 nm, t_R=6.3 min) of the unknown peak in goldfish aqueous humour

Fish size	*n*	Normal goldfish	*n*	Black Moor goldfish
Small	6	0.002±0.004	11	0.018±0.004
Medium	7	0.002±0.004	7	0.020±0.004
Large	6	0.002±0.006	6	0.022±0.000

Table 3
Peak height (absorbance 290 nm, t_R=6.3 min) of the unknown compound in goldfish sera

Fish size	*n*	Normal goldfish	*n*	Megalophthalmic goldfish
Small	6	0.025±0.001	6	0.035±0.005
Medium	4	0.021±0.003	4	0.028±0.003
Large	6	0.015±0.009	8	0.026±0.010

megalophthalmic goldfishes was not statistically significant (Table 3).

3.3. Absence of the compound with an absorption maximum at 290 nm in human and rat sera

The unknown compound observed in fish serum is absent in the animal species we had tested. As shown in Fig. 4, it did not appear in the 290 nm chromatogram of human and rat sera. The major peak in human and rat serum noticeable here has retention time and absorption spectrum (data not shown) identical to that of uric acid. Ascorbic acid is the major peak in the aqueous humour of human eye [7]. Ascorbic acid has an absorbance maximum at 265 nm and is not seen in the 290 nm chromatogram shown in Fig. 4 [7].

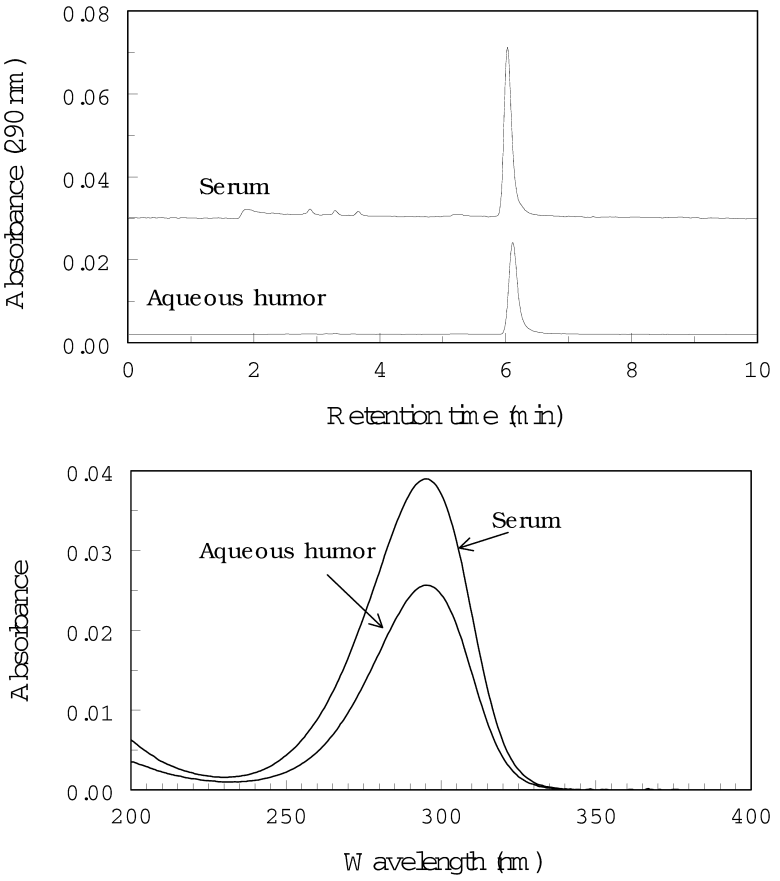

Fig. 3. (a) Comparison of the 290 nm chromatograms of aqueous humour and serum of the same goldfish. (b) Comparison of UV absorption spectra of the compound in the aqueous humour and serum.

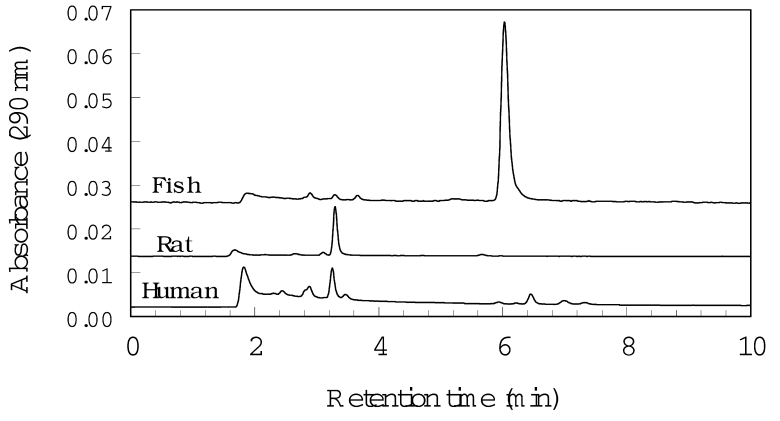

Fig. 4. Comparison of the 290 nm chromatogram of goldfish, human and rat sera.

Table 4
The albumin concentration (mg/ml) in goldfish aqueous humour

Fish size	*n*	Normal goldfish	*n*	Megalophthalmic goldfish
Small	5	0.03±0.01	13	1.27±1.1
Medium	5	0.04±0.00	10	1.83±2.5
Large	6	0.08±0.03	6	2.41±0.9

3.4. Blood–aqueous humour barrier in megalophthalmic eye

The possible breakdown of blood–aqueous humour barrier was examined by analysing the protein concentration. Among serum proteins, a small amount of albumin is normally present in the aqueous humour. Therefore, the amount of albumin in aqueous humour can be used as an indicator of the intactness of the blood–aqueous humour barrier. As shown in Table 4, there was a significant elevation of albumin concentration in the aqueous humour of megalophthalmic goldfish compared to that of the normal goldfish. Also, as the eyeball of the Black Moor goldfish gets larger, the amount of albumin in its aqueous humour increases slightly. The aqueous humour albumin concentration in the eye of the largest megalophthalmic goldfish was 2.41 mg/ml, which is still much lower than that in the blood (40 mg/ml).

In spite of a small leakage of albumin from the blood into the anterior chamber, the majority of the small molecules in the blood as inferred from the 215 nm chromatogram, cannot enter the anterior chamber. If the unknown compound detected by 290 nm absorption in the aqueous humour resulted from leakage from the blood, the unknown compound must be more permeable than the other small molecules in the blood.

4. Discussion

In our previous studies on the biochemical content of aqueous humour, we used wavelengths 215 nm and 265 nm to monitor the eluent in HPLC. The absorption maximum of ascorbic acid is 265 nm, while other compounds in aqueous humour absorb light at 215 nm [7]. The major biochemical content in human [7] and most mammalian eyes [8–10] is ascorbic acid. A small amount of uric acid is noticeable in human aqueous humour at a retention time of 3.5 min because uric acid is the catabolic end product of nucleic acid in human tissues. Nocturnal animals have negligible amounts of aromatic compounds in the aqueous humour [9]. The aqueous humour of normal goldfish is similar to that of nocturnal animals. Only a group of compounds with low retention times was noticeable in the 210 nm chromatogram. These compounds have high absorption at low wavelength (below 220 nm). Ascorbic acid is absent in the aqueous humour of Black Moor goldfish. A remarkably large amount of an unknown compound was observed in the 290 nm chromatogram of all megalophthalmic eyes of Black Moor goldfish. This compound is absent in the normal goldfish eye as well as the eye of rabbits [10], chickens [8] human and rats.

The compound is clearly different from ascorbic acid based on light absorption maximum and retention time. Naturally occurring biochemical compounds with maximal light absorption near 290 nm include uric acid and vitamin E. Vitamin E is a lipid and is not eluted from the column by ammonium phosphate. The retention time of uric acid is 3.5 min. which is much lower than that of the unknown compound observed in the megalophthalmic eye. Furthermore, uric acid has light absorption maximum at 230 nm and 290 nm. This is quite different from the single absorption maximum of the unknown compound (Fig. 2). Aromatic amino acids such as tyrosine and tryptophan has absorption maximum at 220 and 280 nm and low retention time (2–3 min) on the column. So far, we have been unable to identify a known physiological compound that matches the properties of the unknown compound observed in the megalophthalmic goldfish eye. The unknown compound may be the result of an unusual accumulation of either an intermediary metabolic product of amino acids or nucleic acids. A vigorous investigation is needed to identify its chemical structure.

The absence of this compound in the other animal species that we had studied indicates a specific systemic metabolic variation in Black Moor goldfish. The unknown compound is present in the sera of both normal and megalophthalmic goldfishes. However, significant accumulation of this compound only

occurs in the aqueous humour of Black Moor goldfish. It is possible that any effects resulting from this metabolic variation are limited to the eye tissues of the megalophthalmic eye. It has been proposed that the gross enlargement of the Black Moor goldfish eye were driven by high intraocular pressure [11,12]. However Yew et al. [13] were unable to detect any elevated intraocular pressure at different age groups in this strain of goldfish. Efforts are currently underway to ascertain if this biochemical alteration is present in other fish species.

Future investigations on this unique biochemical variation in the megalophthalmic mutant of Black Moor goldfish may lead to an important understanding of the metabolic variation involving the expansion of the eye in this strain of goldfish.

Acknowledgements

The authors are grateful for the technical assistance of Joesphine Ngai.

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