
Ornithine ketoacid aminotransferase in the bovine eye

Seiji Hayasaka, Takashi Shiono, Yoichi Takaku, and Katsuyoshi Mizuno

Ornithine ketoacid aminotransferase in bovine ocular tissues was studied biochemically. The retinal pigment epithelium, ciliary body, iris, and neuroretina showed high specific activity. The cornea and choroid revealed a lower activity. Aqueous humor, lens, vitreous body, and sclera showed no activity. The pH optima of the enzyme in the retinal pigment epithelium and ciliary body were near 8.0.

Key words: ornithine ketoacid aminotransferase, bovine eye, gyrate atrophy of the choroid and retina, retinal pigment epithelium, ciliary body, iris

Ornithine ketoacid aminotransferase (EC 2.6.1.13) catalyzes the interconversion of ornithine and glutamic- γ -semialdehyde, with concomitant interconversion of α -ketoglutarate and glutamate. The enzyme is reported to be deficient in cultured fibroblasts or phytohemagglutinin-stimulated lymphocytes of patients with gyrate atrophy of the choroid and retina.¹⁻⁴ Although patients with gyrate atrophy have an increase of plasma ornithine concentrations,⁵⁻⁹ the most affected and characteristic findings are observed only in the ocular fundus. The enzyme in ocular tissues has not been reported. We therefore examined whether or not ornithine ketoacid aminotransferase activity could be found in bovine ocular tissues.

Materials and methods

Animals and tissue preparation. For one experiment, about 40 eyes, blood, and a piece of liver of

Table I. Ornithine ketoacid aminotransferase activity in bovine ocular tissues, blood, and liver

Tissues	Specific activity*
Cornea	14 \pm 9
Aqueous humor	0
Lens	0
Iris	96 \pm 9
Ciliary body	107 \pm 17
Vitreous body	0
Neuroretina	71 \pm 17
Retinal pigment epithelium	113 \pm 24
Choroid	14 \pm 5
Sclera	0
Blood	0
Liver	12 \pm 5

*nmol of pyrroline-5-carboxylate formed per 30 min per milligram of protein.

Mean \pm S.D. of five experiments.

From the Department of Ophthalmology, Tohoku University School of Medicine, Sendai, Miyagi, Japan.

Supported in part by grants from the Ministry of Education and the Ministry of Health and Welfare, Japan.

Submitted for publication Sept. 17, 1979.

Reprint requests: Seiji Hayasaka, Department of Ophthalmology, Tohoku University School of Medicine, Sendai, Miyagi 980, Japan.

adult cows were maintained at 4° C from the time of slaughter. The aqueous humor was collected by a syringe with a 27-gauge needle. The cornea, iris, ciliary body, lens, vitreous body, neuroretina, retinal pigment epithelial cells, choroid, liver, and blood were dissected as described previously¹⁰ in ice-cold 250 mM sucrose solution containing 20 mM potassium phosphate buffer (pH 8.0). Tissue homogenization was performed in a Waring Blendor. Male Wistar rats were killed by decapitation. The eyes, liver, and blood were collected

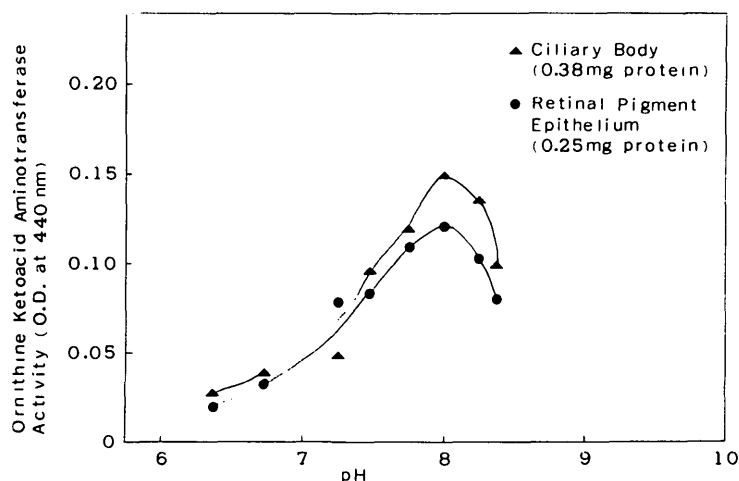


Fig. 1. Effect of pH on ornithine ketoacid aminotransferase activity. The homogenate was incubated at 37° C for 30 min with ornithine (4 μ mol), α -ketoglutarate (2 μ mol), and pyridoxal phosphate (40 nmol), and 0.04 ml of 0.5M phosphate buffer in a total volume of 0.4 ml.

Table II. Ornithine ketoacid aminotransferase activity in adult rat tissues

Tissues	Specific activity*
Liver	242 \pm 41
Blood	0
Retina and choroid	97 \pm 9

*nmol of pyrroline-5-carboxylate formed per 30 min per milligram of protein.

Mean \pm S.D. of four experiments.

immediately. The homogenate of the retina and choroid, liver, and blood were prepared as described above.

Enzyme assay. L-Ornithine hydrochloride was obtained from Nippon Rikagakuyakuhin Co., Tokyo. α -Ketoglutarate, pyridoxal phosphate, and 2-aminobenzaldehyde were obtained from Sigma Chemical Co., St. Louis, Mo. Ornithine ketoacid aminotransferase activity was assayed by a modification of the method of Katsunuma et al.¹¹ The reaction mixture usually contained 2 μ mol α -ketoglutarate, 4 μ mol ornithine hydrochloride, enzyme (homogenate), 40 nmol pyridoxal phosphate, and 0.04 ml of 0.5M phosphate buffer (pH 8.0) in a total volume of 0.4 ml. Incubation was carried out at 37° C for 30 min and terminated with 0.2 ml of saturated 2-aminobenzaldehyde in 1N HCl solution; the samples were immersed in boiling water for 5 min to allow the development of color and then centrifuged. The absorbance at 440 nm in the

clear supernatant was measured against a tissue blank containing trichloroacetic acid. A molar extinction coefficient of 2.71×10^3 was used to calculate the amount of pyrroline-5-carboxylate formed.¹² The 30 min incubation time used throughout the study was within the linear range, and the reaction was proportional to protein concentration. Protein content was determined by the method of Lowry et al.,¹³ with bovine serum albumin as standard. Specific activity is expressed as pyrroline-5-carboxylate formed per 30 min per milligram of protein.

Results

Ornithine ketoacid aminotransferase activity in homogenates of bovine ocular tissues is shown in Table I. The retinal pigment epithelium, ciliary body, iris, and neuroretina showed a high specific activity. Specific activity of the enzyme in the retinal pigment epithelium was about 10-fold higher than in liver. The cornea and choroid demonstrated low specific activity. No activity was observed in aqueous humor, lens, vitreous body, sclera, and blood. As shown in Fig. 1, the optimal pH's of the enzyme in retinal pigment epithelium and ciliary body were near 8.0. The specific activities in homogenates of several rat tissues is shown in Table II. Specific activity in the rat liver was about 20-fold higher than in the bovine liver. The

retina and choroid of the rat showed approximately the same activity as the bovine retina and choroid. No activity was observed in the rat blood.

Discussion

The value obtained for the enzyme activity in the rat liver in our experiments was similar to that in rat liver determined by other investigators.^{11, 14, 15} Therefore it is unlikely that our assay method was incorrect. The enzyme is widely distributed in various tissues of the rat.¹⁶ Particularly, the kidney, liver, and small intestine show high activity in the rat.¹⁶ The enzyme activity in the rat is altered by age, sex, hormones, and diet.¹⁴⁻¹⁶ The enzyme activity in the human liver also has been reported to be dependent on age.¹⁷ Animals in our experiment were adult male and female cows and adult male rats. All animals had free access to food and water. The enzyme activity in bovine liver was significantly lower than in the rat liver. The activity in human adult liver has also been reported to be lower than that in rat liver.¹⁷ Thus different specific activities reported in the literature may be dependent on species differences rather than on the tissue preparations and enzyme assay methods.

The retinal pigment epithelium, ciliary body, and iris of the cow showed about 10-fold higher activity than bovine liver. It has been shown that patients with gyrate atrophy of the choroid and retina have a deficient activity of ornithine ketoacid aminotransferase in their fibroblasts and lymphocytes.¹⁻⁴ Takki⁶ studied patients by fluorescein angiogram and electro-oculogram and suggested that the primary lesion in gyrate atrophy might be at the level of pigment epithelium. However, it remains unclear whether or not the enzyme activity is deficient in the retinal pigment epithelium of the affected patient. The present experiment would suggest a strong correlation between the observation of Takki and the high enzyme activity in the retinal pigment epithelium, implying that the enzyme deficiency could account for the pathogenesis of gyrate retinohoroidal atrophy. In addition, it is of interest that the ciliary body and

iris showed high specific activity of ornithine ketoacid aminotransferase. Some patients with gyrate atrophy have been demonstrated to have myopia,⁶ cataract,⁶ and lens dislocation.⁹ Therefore it is likely that there might be a correlation between the pathogenesis of the complications and the enzyme deficiency in the ciliary body and iris. Cycloscopical observation¹⁸ is thus indicated for gyrate atrophy patients.

We thank Prof. N. Katsunuma, Department of Enzyme Chemistry at Tokushima University, for helpful suggestions and Miss S. Hirakawa for assistance.

REFERENCES

1. Senger RCA, Trijbels JMF, Brussaard JH, and Deutman AF: Gyrate atrophy of the choroid and retina and ornithine-ketoacid aminotransferase deficiency. *Pediatr Res* 10:894, 1976.
2. Shih VE, Berson EL, Mandell R, and Schmidt SY: Ornithine ketoacid transaminase deficiency in gyrate atrophy of the choroid and retina. *Am J Hum Genet* 30:174, 1978.
3. Kaiser-Kupfer MI, Valle D, and Del Valle LA: A specific enzyme defect in gyrate atrophy. *Am J Ophthalmol* 85:200, 1978.
4. O'Donnell JJ, Sandman RP, and Martin SR: Gyrate atrophy of the retina. Inborn error of L-ornithine:2-oxoacid aminotransferase. *Science* 200:200, 1978.
5. Simell O and Takki K: Raised plasma-ornithine and gyrate atrophy of the choroid and retina. *Lancet* 1:1031, 1973.
6. Takki K: Gyrate atrophy of the choroid and retina associated with hyperornithinaemia. *Br J Ophthalmol* 58:3, 1974.
7. Takki K and Simell O: Genetic aspects in gyrate atrophy of the choroid and retina with hyperornithinaemia. *Br J Ophthalmol* 58:907, 1974.
8. Berson EL, Schmidt SY, and Rabin AR: Plasma amino-acids in hereditary retinal disease. Ornithine, lysine and taurine. *Br J Ophthalmol* 60:142, 1976.
9. Akiya S, Ohsawa M, and Ogata T: The long-term observation of two brothers of gyrate atrophy of the choroid and retina with hyperornithinemia. *Acta Soc Ophthalmol Jpn* 81:310, 1977.
10. Hayasaka S, Hara S, Takaku Y, and Mizuno K: Distribution and some properties of cathepsin B in the bovine eyes. *Exp Eye Res* 26:57, 1978.
11. Katsunuma N, Matsuda Y, and Tomino I: Studies on ornithine-ketoacid transaminase. I. Purification and properties. *J Biochem* 56:499, 1964.
12. Strecker HJ: The interconversion of glutamic acid and proline. II. The preparation and properties of Δ -pyrroline-5-carboxylic acid. *J Biol Chem* 235:2045, 1960.
13. Lowry OH, Rosebrough NJ, Farr AL, and Randall

- RJ: Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**:265, 1951.
14. Riih  NCR and Kekom ki MP: Studies on the development of ornithine-keto acid aminotransferase activity in rat liver. *Biochem J* **108**:521, 1968.
 15. Volpe P, Sawamura R, and Strecker HJ: Control of ornithine-transaminase in rat liver and kidney. *J Biol Chem* **244**:714, 1969.
 16. Herzfeld A and Knox WE: The properties, developmental formation, and estrogen induction of ornithine aminotransferase in rat tissues. *J Biol Chem* **243**:3327, 1968.
 17. Kekom ki MP, Riih  NCR, and Bickel H: Ornithine-ketoacid aminotransferase in human liver with reference to patients with hyperornithinemia and familial protein intolerance. *Clin Chim Acta* **23**:203, 1969.
 18. Mizuno K, Asaoka M, and Muroi S: Cycloscopy and fluorescein cycloscopy of the ciliary process. *Am J Ophthalmol* **84**:487, 1977.

Erratum

In Table III of "Immune reactivity to different retinal antigens in patients suffering from retinitis pigmentosa" by C. J. J. Brinkman, A. J. L. G. Pinckers, and R. M. Broekhuysen (*INVEST OPHTHALMOL VIS SCI* **19**:743, 1980), the p value for Bo-Rho, under Leukocyte migration, should be $p < 0.05$.