Volume 329, number 1,2, 178–182 FEBS 12900 August 1993

Skin L-tryptophan-2,3-dioxygenase and rat hair growth

Isao Ishiguro[a], Junko Naito[b], Kuniaki Saito[a,*] and Yoichi Nagamura[c]

[a]*Department of Biochemistry, School of Medicine, Fujita Health University,* [b]*Department of Biochemistry, Fujita Health University College* and [c]*Department of Clinical Chemistry, School of Health Science, Fujita Health University, Toyoake, Aichi 470-11, Japan*

Received 4 May 1993; revised version received 13 July 1993

We have identified a new enzyme, skin L-tryptophan-2,3-dioxygenase (skin TDO), that catalyzes the degradation of L-tryptophan into formylkynurenine in rats. The rate of this degradation peaks in all rats at 5 to 6 weeks after birth, and also, among rats depilated at 8 weeks old, at 10 to 11 weeks after birth. We have also observed that the properties of this enzyme are closer to those of hepatic L-tryptophan-2,3-dioxygenase (hepatic TDO) than to indoleamine 2,3-dioxygenase. Although an intraperitoneal injection of L-tryptophan increased the activity of skin TDO to approximately 2.2 times greater than control values, an intraperitoneal injection of hydrocortisone and α-methyl-DL-tryptophan, both compounds known to affect hepatic TDO activity, had no effect on skin TDO activity. The molecular weight of skin TDO was estimated to be 16.0 kDa, which is close to the molecular weight of hepatic TDO, yet a much larger molecule than indoleamine-2,3-dioxygenase. Increased hair growth rates paralleled increased levels of skin TDO activity in 5- to 6-week-old rats, and marked increases in the activity of skin TDO occurred 2 or 3 weeks after depilation. Enzyme activity was also greatest 2 days before the time of maximum hair root length. Therefore, skin TDO may play an important role in the initiation or suppression of rat hair growth.

L-Tryptophan-2,3-dioxygenase; L-Kynurenine; Skin; Hair growth

1. INTRODUCTION

Hepatic L-tryptophan-2,3-dioxygenase (hepatic TDO; EC 1.13.11.11), is an enzyme that oxidatively degrades L-tryptophan (L-TRP) into formylkynurenine, and is believed to be present only in the liver [1]. Hepatic TDO can be induced by the administration of hormones such as corticosteroids [2]. Indoleamine-2,3-dioxygenase (EC 1.13.11.17) is an enzyme analogous to hepatic TDO in that it also converts L-TRP into formylkynurenine. Indoleamine-2,3-dioxygenase is found in various tissues and is induced by common immune stimulants such as interferon-γ [2–4]. We have identified a third enzyme, skin L-tryptophan-2,3-dioxygenase (skin TDO) that catalyzes the degradation of L-TRP into formylkynurenine in rat skin during specific stages of rat development [5]. Formylkynurenine is then metabolized into L-kynurenine (L-KYN) by formamidase, the activity of which is extremely high. Therefore, L-KYN is the apparent product of hepatic TDO or indoleamine-2,3-dioxygenase. In 1960, Ishiguro et al. reported that, unlike the hair of other animals, rat hair contains 3–6 mg of L-KYN per gram of the hair [6]. L-KYN is a metabolite of L-TRP that is also a precursor for the formation of several physiologically important substances such as NAD. The mechanisms responsible for L-KYN accumulation in rat hair and its biological significance, however, are uncertain. The purpose of the present study was to examine the biochemical properties of rat skin TDO and to compare these to hepatic TDO and indoleamine-2,3-dioxygenase. Further, this study also sought to examine a possible link between skin TDO and rat hair growth.

Correspondence address: I. Ishiguro, Department of Biochemistry, School of Medicine, Fujita Health University College, Toyoake, Aichi 470-11, Japan.

**Present address:* Laboratory of Clinical Science, Building 10, Room 3D40, National Institute of Mental Health, Bethesda, MD 20892, USA.

Abbreviations: L-KYN, L-kynurenine; L-TRP, L-tryptophan; skin TDO, skin L-tryptophan-2,3-dioxygenase; hepatic TDO, hepatic L-tryptophan-2,3-dioxygenase; αMT, α-methyl-DL-tryptophan.

2. MATERIALS AND METHODS

Three to 12-week-old male Sprague–Dawley and Wistar rats (SCL Ltd., Hamamatsu, Japan) were used throughout. Animals were kept on a 12 h:12 h light–dark cycle and were maintained ad libitum on a cubed diet of Oriental MF (Oriental Yeast Ltd., Tokyo, Japan) and water.

L-TRP, L-KYN, 3-hydroxy-DL-kynurenine, α-methyl-DL-tryptophan (αMT), hematin, catalase, cycloheximide, and hydrocortisone phosphate were obtained from Sigma Chemical Co. (St. Louis, MO). Glucose-6-phosphate, glucose-6-phosphate dehydrogenase, NADP, and Combithek (calibration proteins for chromatography) were obtained from Boehringer-Mannheim Biochemicals (Tokyo, Japan). Cellulofine GCL-1000-m was obtained from Biochemical Industry (Tokyo, Japan). All other reagents were of the purest grade available from standard suppliers.

Rats that had been thoroughly depilated using Evacream hair removal compound were anesthetized. Pieces of skin were excised from the upper back, frozen in liquid nitrogen for 50 s, and then broken into small pieces. Skin samples were homogenized 3 times for 30 s each using a Polytron homogenizer with 9 vols. (per gram of tissue) of

 Published by Elsevier Science Publishers B.V.

ice-cold 0.25 M sucrose. To measure the length of the hair, including the hair root buried under the skin, we used hair dye. After plucking, the undyed parts of the hair were separated with scissors from the whole hair, and were used as the hair root. Other experimental details and the assays for skin TDO and L-KYN quantification have been described previously [5–7]. Enzyme activities were calculated from the concentration of L-KYN obtained after incubation with L-TRP and were expressed as L-KYN formed in μmol/g (wet tissue)/h or in μmol/ml (supernatant)/h.

The HPLC equipment used for quantifying the enzyme activity consisted of a Yanaco liquid chromatograph, Model L-3200 (Kyoto, Japan) with a Yanapak ODS-H column (4.6 × 250 mm), and an Eicom electrochemical detector, Model ECD-100 with a graphite electrode (Kyoto, Japan). Chromatography was carried out with 0.1 M sodium phosphate buffer, pH 3.1, containing 10 μM EDTA as mobile phase with a flow rate of 1.4 ml/min. The column was kept in an oven heated to 25°C, and the detector was operated at a voltage of 300 mV.

3. RESULTS AND DISCUSSION

The activity of skin TDO in rats changes depending on their growth stages, and a remarkable seasonal variation of the enzyme activity was also observed (Fig. 1). In the summer, the highest activity of skin TDO was observed in 5-week-old rats, but this activity was only about 25% of the activity observed in 6-week-old rats in the winter. Moreover, the marked increases in the activity of skin TDO observed 2 or 3 weeks after depilation were also found to be lower in the summer than in the winter. Since the animals were kept in a temperature-controlled environment, the low activity of skin TDO in the summer cannot be explained by seasonal temperature changes. This phenomenon was observed over a 3 year period, yet its mechanism remains to be established. No corresponding variations have been observed with respect to the activities of hepatic TDO or extrahepatic indoleamine-2,3-dioxygenase.

In 6-week-old rats in the winter, the activity of skin

Table I

L-Kynurenine content in several kinds of rat hair

Strains contents	Hair colors	L-Kynurenine (mmol/g of hair)
Wistar	albino	22.6 ± 2.8
DA	agouty (wild color)	22.3 ± 3.1
HO[a]	black	33.4 ± 2.9
	albino	29.1 ± 3.3
HO[b]	dark brown	14.6 ± 0.7
	albino	15.2 ± 0.8
RSC/N-p/p[c]	light brown	14.5 ± 0.7
	albino	25.7 ± 1.9

[a] The head and shoulder are black.
[b] The body is partially dark brown.
[c] The body is partially light brown.
Rat hairs of the dorsal posterior region (unless otherwise stated) were plucked and washed with 50% ethanol and then dried thoroughly with filter paper before analysis. L-KYN in each sample (20 mg) was then extracted with 2.0 ml of 0.01 N HCl at 80°C for 15 min and was quantified according to the previously described enzyme–HPLC method [7]. Values are the means ± S.E.M. consisting of duplicate samples from 3 independent experiments.

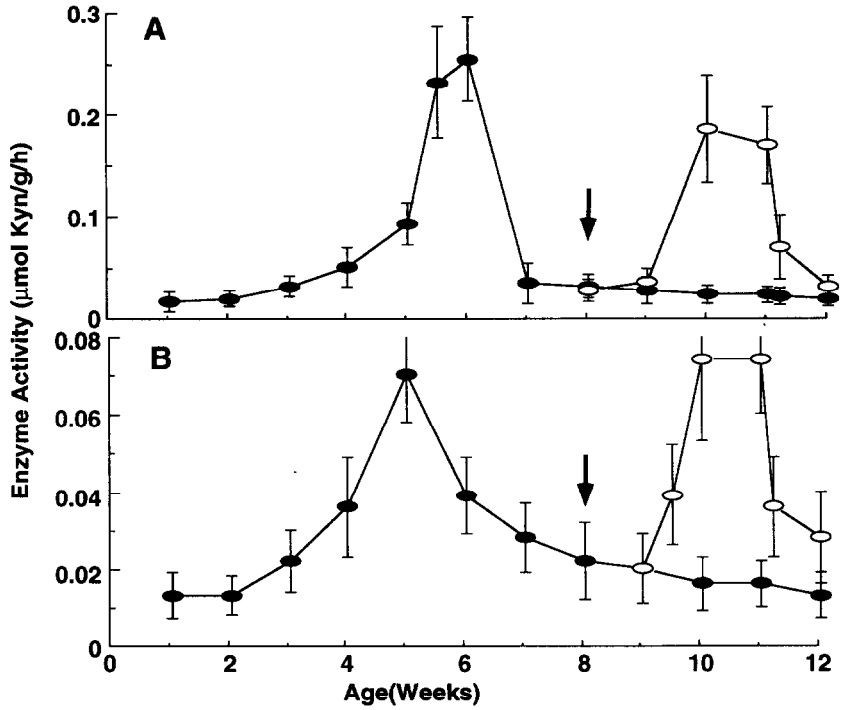

Fig. 1. Skin L-tryptophan-2,3-dioxygenase activity in rat. (A) Winter. (B) Summer. Dorsal posterior region (unless otherwise stated) of rats were used to prepare the homogenates. Arrows show the time of depilation. Skin homogenates of the untreated (●), and depilated (○) rats. Data are the mean ± S.E.M. consisting of duplicate samples from 4 independent experiments. Part of Fig. 1 has been reported in the Proceedings of the 6th Meeting of International Study Group for Tryptophan Research [8].

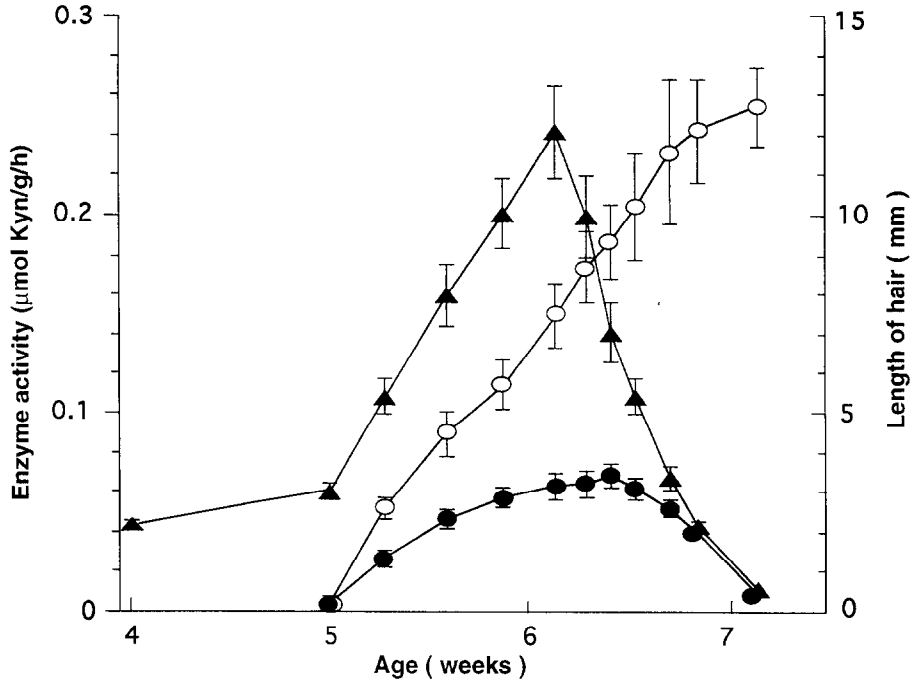

Fig. 2 Relationship between skin L-tryptophan-2,3-dioxygenase activity and growth rate of the hair in rat in the winter. To distinguish the hair which is buried under the skin from the whole part of the hair, a hair-dye method was used, since the buried part was not dyed. Skin TDO activities in the rat, (▲); length of the hair under the skin, (●); full-length of the hair (○). Values are the mean ± S.E.M. consisting of duplicate samples from 4 independent experiments.

TDO was about 20 times higher in the hair root than in the skin homogenate, 5.21 ± 0.08 vs. 0.25 ± 0.02 μmol/ g/h L-KYN. We also observed that the enzyme activity in the skin in which the hair root had been eliminated, decreased to approximately 25% of the activity of the skin that maintained the hair root [5]. These results suggest that much of the activity in the skin homogenate originated in the hair root. Furthermore, the rates of hair growth paralleled the increased levels of enzyme activity at 5–6 weeks of age (Fig. 2). The maximum hair root length was observed 2 days after the time of the greatest enzyme activity, and thereafter, the growth rate decreased precipitously. These results suggest that the induction of skin TDO in the hair root may initiate hair growth and that inactivation of the enzyme may suppress hair growth.

We also observed that among 4- to 8-week-old rats, 6-week-old rats had the highest amount of [^{3}H]L-KYN in their hair 1 h after an intravenous injection of [^{3}H]L-TRP [8]. The activity of hepatic TDO remained constant having no relation to age or to season, unless a large amount of L-TRP (in excess of 500 mg/kg) or hormones had been administered. Therefore, the accumulation of L-KYN in the hair cannot be attributed to hepatic TDO activity. These findings suggest that the L-KYN present in the hair is supplied via skin TDO activity rather than hepatic TDO. The K_m values for skin TDO (1 μM for L-TRP [5]) are smaller than physiological concentration of L-TRP in skin (80 μM). Therefore, skin TDO may also be an important regulatory enzyme for L-KYN formation in the skin.

The physiological role of L-KYN in rat hair remains

Table II

Effects of several compounds on the activity of rat skin L-tryptophan-2,3-dioxygenase in vivo

Additions	Skin TDO activity (μmol L-KYN/g/h)			
	Control (saline)	L-TRP (500 mg/kg)	α-MT (2 mg/kg)	Hydrocortisone (25 mg/kg)
None	0.198 ± 0.021	0.436 ± 0.041	0.217 ± 0.034	0.194 ± 0.023
Cycloheximide (4 mg/kg)	0.103 ± 0.015	0.315 ± 0.035	nd	nd

Six-week old rats in winter were used. All compounds were dissolved in 2.5 ml/rat and injected intraperitoneally to rats 4 h before the experiments. Values are the means ± S.E.M. consisting of duplicate samples from 4 independent experiments. nd, not determined.

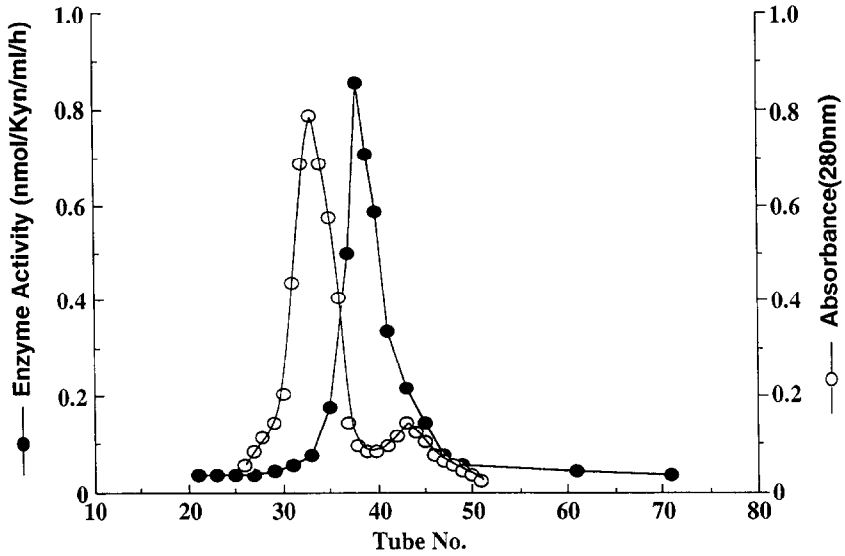

Fig. 3. Column chromatography of rat skin L-tryptophan-2,3-dioxygenase by cellulofine GCL-1000-m. Six-week-old rats in winter were used. All preparations were performed at 4°C. Rat skin was prepared and homogenized with 9 vols. of ice-cold solution containing 50 mM potassium phosphate buffer (pH 7.0), 0.1 mM L-TRP, and 0.1 M KCl by using a Polytron homogenizer. The supernatant fraction (10 ml), obtained from the homogenate by centrifugation at 105,000 × *g* for 60 min, was concentrated 5 times with an Amicon filter. The concentrated crude enzyme solution (1.0 ml) was applied to a cellulofine GCL-1000 column (16 × 700 mm), which had been equilibrated with the solution. The chromatography was performed with the same solution and a flow rate at 0.28 ml/min under the pressure of 0.05 kgf/cm^2. Each fraction (1.5 ml/tube) was collected. Authentic proteins, such as ferritin, catalase, aldolase, bovine serum albumin, ovoalbumin, β-lactoglobulin, chymotrypsinogen A and cytochrome *C* were chromatographed with the same column and conditions in order to calculate the molecular weight of skin TDO. The enzyme activity was quantified as described in the legend to Fig. 1. Skin TDO activities in the fractions (●); absorbance at 280 nm of the fractions (○). Values are the mean ± S.E.M. consisting of duplicate samples from 3 independent experiments.

unclear. Ishiguro et al. have shown that L-KYN is used as a starting material for the formation of hair pigment in albino rats [9]. Because L-KYN is also present in the hair of black and brown rats, it is unlikely that L-KYN is used only for this purpose (Table I). L-KYN is also an important intermediate in the NAD synthetic pathway from L-TRP. Induction of hepatic TDO by L-TRP or hydrocortisone, and inhibition by αMT have been demonstrated [10,11]. Therefore, we examined the effects of these substances on skin TDO. The activity of skin TDO was induced about 2.2 times after intraperitoneal injection of L-TRP (500 mg/kg) compared with that of the control, but the activity did not change after administration of either hydrocortisone (25 mg/kg) or αMT (2 mg/kg) (Table II). Rats were also given an intraperitoneal injection of cycloheximide, a general inhibitor of protein synthesis, to determine whether the activity of skin TDO originates from the synthesis of the enzyme protein. The enzyme activity of saline or L-TRP administrated animals decreased 48% and 27%, respectively. These results indicate that at least a part of skin TDO is synthesized de novo in the rat skin including the hair roots. In addition, rats given daily intraperitoneal injections of L-TRP for 14 days after depilation demonstrated a higher rate of hair growth than control animals given daily intraperitoneal injections of saline (data not shown).

The molecular weight of skin TDO was determined from the experiment of Cellulofine GCL-1000-m column chromatography by using the crude extract as the enzyme and it was estimated to be 16.0 kDa as seen in Fig. 3. This value is close to the molecular weight of hepatic TDO which is 16.7 kDa [12], but is much greater than indoleamine-2,3-dioxygenase which is 4.1 kDa [13].

In conclusion, rat skin TDO activity changes according to the hair growth stages and a remarkable seasonable variation of this enzyme was observed. Skin TDO may have an important role for initiation or suppression of rat hair growth.

Acknowledgements: We appreciate the useful guidance of J.S. Crowley (National Institute of Mental Health). We also wish to thank H. Takahashi (Fujita Health University) for his generous gift of several kinds of rat hair.

REFERENCES

[1] Knox, W.E. (1955) in: Methods in Enzymology (Colowick, S.P. and Kaplan, N.O. eds.) vol. 2, pp. 242–243, Academic Press, New York/London.
[2] Saito, K., Markey, S.P. and Heyes, M.P. (1992) Neuroscience 51, 25–39.
[3] Yoshida, R., Urade, Y., Nakata, K., Watanabe, Y. and Hayaishi, O. (1981) Arch. Biochem. Biophys. 212, 629–637.
[4] Saito, K., Lackner, A., Markey, S.P. and Heyes, M.P. (1991) Brain Res. 540, 353–356.
[5] Naito, J., Ishiguro, I., Nagamura, Y. and Ogawa, H. (1989) Arch. Biochem. Biophys. 270, 236–241.

[6] Hotta, K., Ishiguro, I., Naito, J. and Kuzuya, H. (1960) Seikagaku 32, 28–31.
[7] Naito, J., Ishiguro, I., Murazumi, T. and Morimoto, M. (1987) Anal. Biochem. 161, 16–19.
[8] Naito, J., Ishiguro, I., Nagamura, Y. and Ogawa, H. (1991) in: Kynurenine and Serotonin Pathways (Schwarcs, R. et al. eds.) pp. 527–529, Plenum Press, New York.
[9] Ishiguro, I. and Nagamura, Y. (1972) Seikagaku 44, 22–31.
[10] Knox, W.E. and Mehler, A.H. (1951) Science 113, 237–240.
[11] Oravec, M. and Sourkes, T.L. (1970) Biochemistry 9, 4458–4464.
[12] Schutz, G. and Feigelson, P. (1972) J. Biol. Chem. 247, 5327–5332.
[13] Shimizu, T., Nomiyama, S., Hirata, F. and Hayaishi, O. (1978) J. Biol. Chem. 253, 4700–4706.