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ORIGINAL ARTICLE

# Identification of L-3-hydroxykynurenine *O*-sulfate in the buccal gland secretion of the parasitic lamprey, *Lethenteron japonicum*

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**Abstract** Parasitic lampreys are known to secrete proteins having anticoagulant and vasodilator activities from the buccal glands during feeding on their host's blood. However, small molecules in the secretion have never been explored in detail. We examined the secretion of Japanese liver lamprey (*Lethenteron japonicum*) for small molecules and found an intensely fluorescent substance upon gel filtration. After purification by anion-exchange chromatography and reversed-phase HPLC, structure of the compound was determined to be L-3-hydroxykynurenine *O*-sulfate by NMR- and UV-spectrometry, complemented with enzymatic and chemical degradation. In vertebrates, the sulfate ester of 3-hydroxykynurenine is a compound that has been regarded as a urinary metabolite of tryptophan but not reported from normal tissues to date. Although the function of this molecule in the buccal glands remains to be elucidated, it is remarkable that the same substance was described in 1960s from two species of blood-sucking insects, *Rhodnius prolixus* and *Triatoma infestans*, suggesting its potential role in blood-feeding.

**Keywords** 3-Hydroxykynurenine · 3-Hydroxykynurenine *O*-sulfate · Lamprey · Buccal gland · *Lethenteron japonicum*

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**Abbreviations**

| | |
|---|---|
| 3Hyk | 3-Hydroxykynurenine |
| 3Hyk($SO_3H$) | 3-Hydroxykynurenine *O*-sulfate |
| 5Hyk | 5-Hydroxykynurenine |
| BG360 | Buccal gland small molecule |
| FDAA | 1-Fluoro-2,4-dinitrophenyl-5-L-alanine amide (Marfey's reagent) |
| Kyn | Kynurenine |
| MCA | 4-Methylcoumaryl-7-amide |
| Tyr($O_3H$) | Tyrosine *O*-sulfate |

## Introduction

Exocrine glands of venomous and parasitic animals are rich sources of bioactive substances and have been a subject of intense investigation for biochemical and pharmacological interest (for recent reviews, see Fox and Serrano 2007; Yamazaki and Morita 2007; Lehane 2005; Corral-Rodríguez et al. 2010; Vetter et al. 2010). Particularly, much attention has been paid to the salivary glands of blood-feeding invertebrates including leeches, mosquitoes, and ticks (Lehane 2005; Corral-Rodríguez et al. 2010; Andersen 2010). Besides hirudin, a pharmaceutically successful thrombin inhibitor of medicinal leech (*Hirudo medicinalis*), a vast number of inhibitors against blood coagulation factors were isolated from the salivary glands of these animals as potential anti-thrombotic agents, which are compiled in the inhibitor section of the MEROPS peptidase database (Rawlings et al. 2010). Almost all of the hematophagous animals are invertebrates and only lampreys and vampire bats are true vertebrate blood feeders (Hopla et al. 1994). Parasitic lampreys have a pair of well-developed exocrine buccal glands open to the oral cavity. More than 80 years ago, it was shown that these lampreys

secrete anticoagulant called lamphredin from the buccal glands (Gage and Gage-Day 1927), but only recently has its fibrinogenolytic activity become evident (Xiao et al. 2007). Since lampreys, together with hagfishes, represent the most ancient group of vertebrates with a fossil record dating back to at least 360 million years ago (Gess et al. 2006), it was expected that lampreys had independently evolved unique molecules for blood-feeding and parasitism. Indeed, we have previously identified a novel L-type calcium channel blocker in the buccal gland secretion of the Japanese river lamprey (*Lethenteron japonicum*, a synonym for *Lampetra japonica*), which seems to act as a vasodilator during blood sucking (Ito et al. 2007). So far, studies on the buccal gland secretion have focused on proteinaceous substances and small molecules in the secretion are not investigated to date. Here, we describe the structural characterization of a fluorescent small molecule having an absorbance maximum at 360 nm, and hence designated as BG360, in the buccal glands of *L. japonicum* and its identification as L-3-hydoxykynurenine *O*-sulfate.

## Materials and methods

### Animals

Lampreys were collected at the Shinano river Ohkouzu floodway, Niigata, Japan, in June. Outer tissues were removed carefully to expose the buccal glands and content of the glands was withdrawn by a microsyringe. Usually 50–100 µl of secretion was obtained from a single animal.

### Isolation of the lamprey small molecule (BG360)

Dense secretion collected from ten animals was diluted fivefold with 10 mM Tris–HCl buffer, pH 8, 0.1 M NaCl, centrifuged to remove insoluble materials, and fractionated by gel filtration on a Sephacryl S-200 HR column (4.5 × 80 cm, GE Healthcare Bio-Science) equilibrated with the same Tris–HCl buffer. Elution was monitored by absorbance at 280 and 360 nm. Fractions (17-ml) absorbing at 360 nm were pooled and lyophilized. They were further purified by anion-exchange chromatography on a Mono-Q or Resource-Q column (1 ml bed volume, GE Healthcare Bio-Science) using a linear gradient of $NH_4HCO_3$ (pH 8) from 10 mM to 1 M. Peak fraction was lyophilized and finally purified by reversed phase HPLC as described below.

### HPLC

Reversed-phase HPLC for analytical and preparative purposes was carried out on an octadecylsilane column (4.6 × 150 mm, ODS-100V, TOSOH). The column was eluted by a linear acetonitrile gradient of 0–30 % in 0.05 % trifluoroacetic acid over 30 min. The flow rate was set at 1.0 ml/min. Peaks were detected by absorbance at 360 nm.

### Amino acid analysis

Samples were analyzed on a cation exchange column (4 × 150 mm) in a Hitachi 835 amino acid analyzer modified to elute with 0.2 M sodium citrate buffer, pH 4.9, containing 1 M NaCl at a flow rate of 0.225 ml/min. Peaks were detected by absorbance at 570 nm after ninhydrin reaction.

### Spectroscopic analyses

UV-absorption spectra were recorded at pH 1.5 (0.033 M HCl), pH 7.0 (0.1 M sodium phosphate buffer), and pH 13 (0.1 M NaOH) by a Shimadzu spectrophotometer (Model UV-1650PC). Mass spectrum was recorded on a JEOL SX-100 mass spectrometer by fast atom bombardment ionization using glycerol as a matrix in the negative-ion mode. The sample for NMR measurement was exchanged twice with $^2H_2O$ at room temperature with immediate lyophilization and finally dissolved in $^2H_2O$ (99.999 atom % $^2H$) and analyzed at 25 °C on a Varian Inova 600 MHz NMR spectrometer. Chemical shifts ($\delta$) are expressed by reference to internal acetone ($\delta = 2.218$ ppm).

### Enzymatic and acid hydrolysis of sulfate ester

Arylsulfatase digestion: sample (ca 4 nmol) was digested with sulfatase from keyhole limpet (0.2 unit, Sigma-Aldrich) in 200 µl of 0.1 M sodium acetate buffer, pH 5, for 24–48 h at 30 °C. An equal amount of the sample was also incubated in the same buffer without enzyme. Digests were analyzed by reversed-phase HPLC. Acid hydrolysis: samples were exposed to the vapor of 5.7 N HCl for 1 h at 110 °C under vacuum.

### Analysis of sulfate and phosphate

Ion chromatography on the Mono-Q anion-exchanger column (1 ml) was used to discriminate between sulfate ester and phosphate ester. Inorganic ions were eluted from the column under isocratic conditions with 4.0 mM potassium hydrogen phthalate (adjusted to pH 6.0 with KOH) at 35 °C and a flow rate of 1 ml/min. Ions were measured by indirect UV detection (vacancy) at 285 nm, which can detect a substance lacking absorbance as a negative peak against the UV-absorbing background of the phthalate buffer (Denkert et al. 1981; Heisz 1983). Tyrosine *O*-sulfate ($Tyr(O_3H)$) synthesized according to the method of Tallan et al. (Tallan et al. 1955) was hydrolyzed with 5.7 N HCl and used to validate the method.

### Paper chromatography

Ascending chromatograms were developed on Whatman No. 1 chromatography paper with the organic phase of 1-butanol-acetic acid–water (4:1:5, by volume) (Dalgliesh 1952). Amino acids were detected by fluorescence under UV light (365 nm).

### Determination of stereochemistry

Enantiomeric configuration was assigned by derivatization with Marfey's reagent, 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (FDAA, Marfey 1984). 3-Hydroxykynurenine (3Hyk) and acid hydrolysate of BG 360 were reacted with FDAA (Sigma-Aldrich) in a mixture of triethylamine and acetonitrile (2:3, by volume) for 60 min at 40 °C (Scaloni et al. 2003). The derivatives were analyzed by reversed-phase HPLC using an 80-min linear gradient of acetonitrile from 0 to 60 % in 0.05 % TFA. Peaks were detected by absorbance at 340 nm. Since only racemic 3Hyk (Tokyo Chemical Industry) was commercially available, L-enantiomer was prepared as follows: About 200 nmol of the racemic 3Hyk was *N*-acetylated with 1 mmol of acetic anhydride in 50 µl of 2 M sodium acetate buffer, pH 5.5. Acetyl 3Hyk was isolated by HPLC and digested with acylase (100 µg, 0.5 unit/mg, Acylase Amano, Amano Enzymes) in 50 mM Tris–HCl, pH 8.0, 1 mM $CoCl_2$ at 37 °C for 60 min. L-3Hyk was isolated by HPLC.

### Inhibition of blood coagulation factors

Activity of thrombin and factor Xa (bovine, Sigma) was measured with 0.1 mM Boc–Val–Pro–Arg–MCA and Boc–Ile–Glu–Gly–Arg–MCA (peptidyl 4-methylcoumaryl-7-amides, Peptide Institute), respectively, in 50 mM Tris–HCl, pH 8.0, 0.005 % Triton X-100 at 30 °C, in the presence and absence of ca 1 mM BG360. Aliquots were withdrawn at appropriate time intervals and released; 7-amino-4-methylcoumarine was quantified by the absorbance at 340 mm on an octadecylsilane column (4.6 × 35 mm, Capcell Pak C18, Shiseido). A 20-min linear acetonitrile gradient from 0 to 75 % in 0.05 % trifluoroacetic acid at a flow rate of 1 ml/min was used for elution.

## Results

### Isolation of BG360 from buccal gland secretion

Gel filtration chromatography of the buccal gland secretion (Fig. 1) yielded a very large peak fraction absorbing at 360 nm eluted far behind two major protein peaks I and II, which corresponded to lamprey plasma albumin and an L-type calcium channel blocker, respectively (Ito et al. 2007). This small molecule, tentatively designated as BG360, was further purified by chromatography on the Mono-Q anion-exchanger column (not shown) and finally by reversed-phase chromatography on an octadecylsilane column (Fig. 1b). Lyophilized BG360 showed bright blue–white fluorescence under the UV light around 360 nm.

### Spectrometric characterization of BG360

The molecular mass of BG360 was determined to be 304.0 Da by mass spectrometry based on the molecular ion peak observed at m/z 303.0 ($[M–H]^-$) in the negative ion mode (Fig. 2). BG360 showed a characteristic UV absorption spectrum, having maxima at 362, 260, and 225 nm at pH 7 (Fig. 3a). We searched for similar spectra in the literature and found its close resemblance to those of

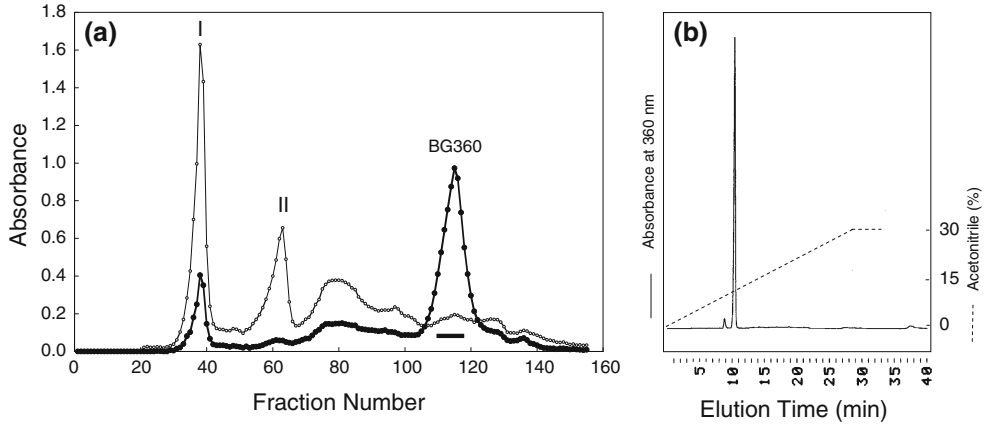


**Fig. 1** Gel filtration of buccal gland secretion of the Japanese river lamprey, *Lethenteron japonicum* (**a**) and reversed phase HPLC of the purified BG360 (**b**). **a** Secretion was fractionated by gel filtration on a Sephacryl S-200 HR column. *Open circles* absorbance at 280 nm, *solid circles* at 360 nm. Fractions containing BG360 indicated by the *bar* were pooled for further purification on anion-exchange chromatography. **b** Reversed-phase HPLC of BG360 on an octadecylsilane column

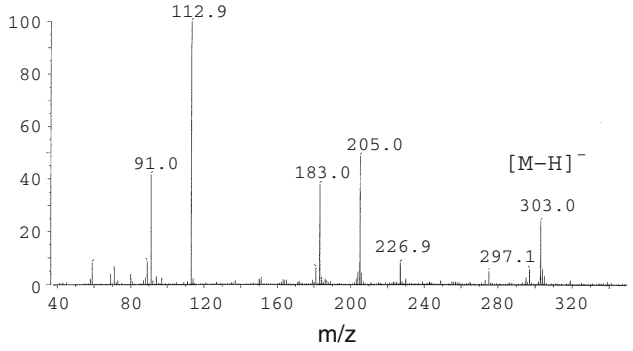


**Fig. 2** FABMS analysis of BG360 isolated from the lamprey buccal glands. Ions were recorded in the negative ion mode using glycerol as a matrix. The molecular ion peak is observed at m/z 303. Peaks at m/z 183.0 and 91.0 are derived from the matrix

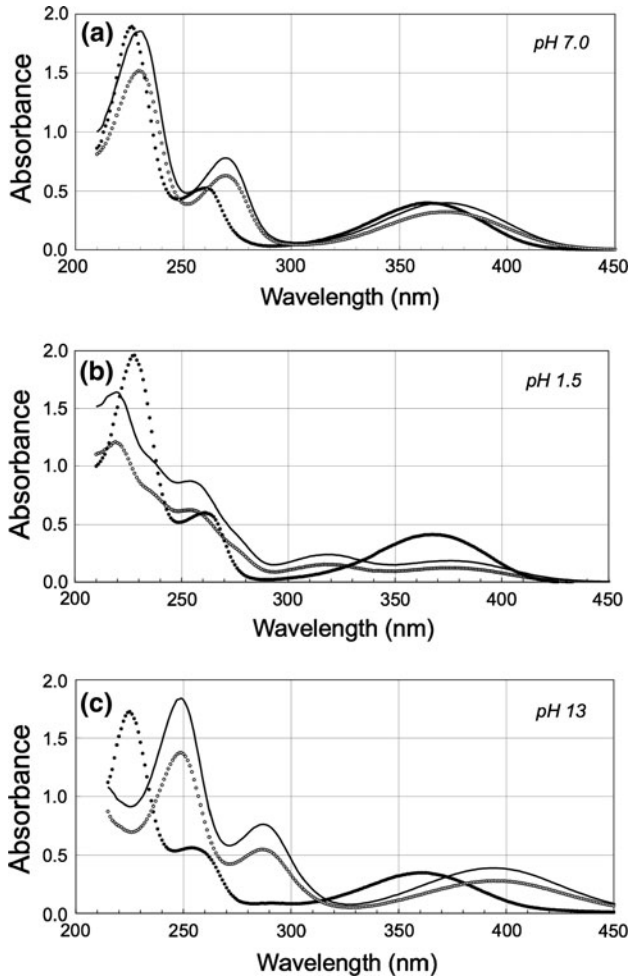


**Fig. 3** UV spectra of BG 360 at different pHs. Absorption spectra were recorded at **a** pH 7.0 (0.1 M sodium phosphate buffer), **b** pH 1.5 (0.033 M HCl), and **c** pH 13 (0.1 M NaOH). *Solid circles* BG 360, *open circles* acid hydrolysate of BG360, *solid line* 3-Hyk (0.1 mM)

kynurenine (Kyn, $M_r = 208$) and 3-hydroxykynurenine (3Hyk, $M_r = 224$) (Viscontini and Schmid 1963; Atherton et al. 1993). However, the molecular mass of BG360 is larger than Kyn and 3Hyk by 96 and 80, respectively, suggesting a kynurenine derivative substituted at the aromatic ring by sulfate or phosphate, that is, a sulfate or phosphate ester of hydroxykynurenine, since these acids are isobaric. Acid hydrolysis of BG360 yielded a peak corresponding to authentic 3Hyk on the reversed-phase HPLC (Fig. 4a). When BG360 and its acid hydrolysate were analyzed by the cation-exchange column of the amino acid analyzer at pH 4.9, BG360 was not retarded to the column indicating its strongly acidic nature (not shown), while the hydrolyzed BG360 was coeluted with 3Hyk (Fig. 4b). Hydroxykynurenine structure was further confirmed by pH dependence of its UV spectrum. Due to the ionizable phenolic hydroxy group, the spectrum of hydroxykynurenine varies widely depending on pH, while those of Kyn and hydroxykynurenine *O*-sulfate are rather insensitive to pH (Viscontini and Schmid 1963; Atherton et al. 1993). UV-spectra of acid hydrolyzed BG360 showed the characteristic pH-dependence of 3Hyk, and that of BG360 was stable in a wide pH range (Fig. 3) as Kyn (not shown). These spectral properties are in accordance with the previous reports (Viscontini and Schmid 1963).

Another possible structure as a tryptophan metabolite is 5-hydroxykynurenine (5Hyk), for which we could not obtain an authentic material (Kido et al. 1967). The $^1$H NMR spectrum of BG360 showed the presence of a pair of aromatic doublets ($\delta = 7.77$ and 7.51 ppm, $J = 7.8$ Hz) and a triplet ($\delta = 6.78$ ppm, $J = 7.8$ Hz), all with *ortho*-couplings suggesting a 1,2,3-substitution, i.e., a 3-hydroxy-derivative. In addition, it was reported that synthetic 5Hyk has two absorption maxima at pH 7.4 (224, 380 nm) and pH 12.0 (240, 400 nm), and three at pH 2.0 (251, 308, 370 nm) (Kido et al. 1967). This is quite different from those of 3Hyk and the BG360 hydrolysate, which exhibited three absorption maxima at pH 7.0 (230, 270, 372 nm) and pH 13 (248, 287, 372 nm), and four at pH 1.5 (219, 260, 318, 377 nm) (Fig. 3). Furthermore, when analyzed by paper chromatography on Whatman No. 1 paper developed with the organic phase of 1-butanol-acetic acid–water (4:1:5, by volume), 5Hyk migrated with a remarkably small $R_F$ (retardation factor) value, only one-half that of 3Hyk ($R_F$: 0.1 vs. 0.2) (Boyland et al. 1956). The acid hydrolysate of BG360 comigrated with standard 3Hyk and there was no spot below it. These results, together with that of NMR spectrometry, confirmed the structure to be 3-hydroxykynurenine.

## Identification of sulfate ester

To discriminate between the isobaric sulfate and phosphate esters, BG360 was digested with keyhole limpet arylsulfatase and analyzed by the reversed phase HPLC. As shown in Fig. 5a, sulfatase digestion yielded a peak

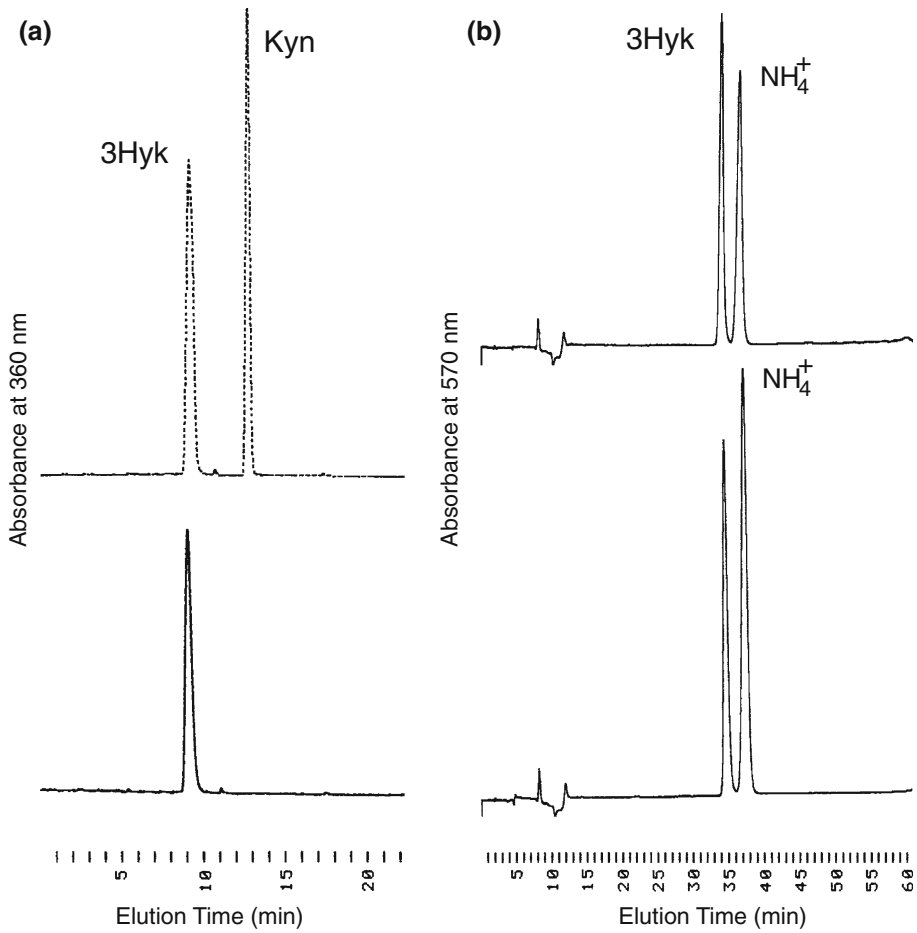


**Fig. 4** Reversed-phase HPLC (**a**) and amino acid analysis (**b**) of the acid hydrolysate of BG 360. **a** *Upper panel* 3-Hyk and Kyn, *lower panel* hydrolysate of BG360. **b** *Upper panel* 3-Hyk, *lower panel* hydrolysate of BG360

corresponding to 3Hyk after 48 h, while BG360 did not change in the absence of the enzyme despite the prolonged time of incubation. To further confirm that digestion was actually by the sulfatase and not by a phosphatase in the enzyme preparation, sulfate liberated by HCl hydrolysis was confirmed by ion chromatography using the Mono-Q anion exchanger and indirect UV detection. Figure 5b shows a complete separation of phosphate and sulfate ions by this system and the unambiguous presence of sulfate ion in the hydrolysate of BG360. A peak corresponding to chloride ion present in the hydrolysate is probably due to a trace of HCl used for hydrolysis, since it is also seen in the hydrolysate of tyrosine *O*-sulfate (Fig. 5b).

## Stereochemistry and structure of BG360

A substoichiometric amount of FDAA to 3Hyk was used to reduce the formation of *N*,*O*-disubstituted derivative, which gave poor resolution on HPLC. *N*-substituted D,L-3Hyk was eluted as well separated two peaks by HPLC (Fig. 6a) and the L-enantiomer prepared by acetylation of D,L-3Hyk followed by acylase digestion was coeluted with the first one (Fig. 6b) in accordance with the notion that many L-amino acids derivatized with FDAA are eluted faster than their (D) counterparts (Scaloni et al. 2003). Marfey's analysis of the acid hydrolysate of BG360 indicated the L-configuration for 3Hyk in BG360 (Fig. 6c). From these spectral and chromatographic data, the structure of BG360 was identified to be L-3-hydroxykynurenine *O*-sulfate (3Hyk($SO_3H$), Fig. 7). The calculated monoisotopic mass is 304.0365 ($C_{10}H_{12}O_7N_2S$). Assuming a molar extinction coefficient identical to that of 3Hyk (4,000 at 360 nm), concentration of 3Hyk($SO_3H$) in the buccal gland was roughly estimated to be around 10 mM (average of three measurements).

## Examination of inhibitory activity on blood coagulation factors

Possible anticoangulating activity of L-3Hyk($SO_3H$) was examined by direct inhibitory activity against bovine α-thrombin and factor Xa using 0.1 mM peptidyl 4-methylcoumarine-7-amide substrates. Because the intrinsic fluorescence of 3Hyk($SO_3H$) interfered with the fluorometric measurement, released 7-amino-4-methylcoumarine was determined by HPLC. No inhibitory activity for these

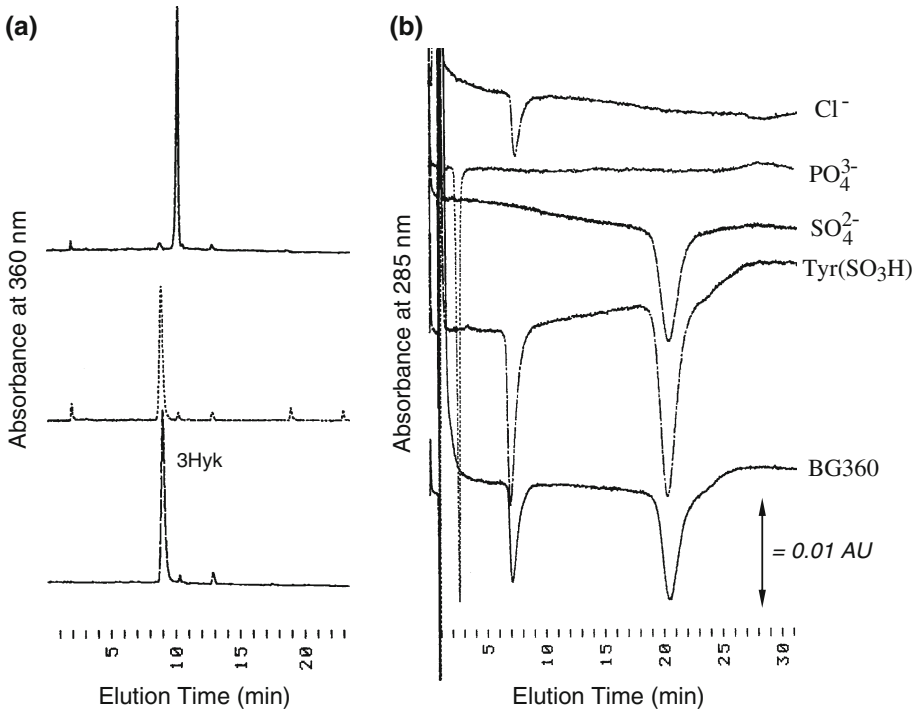


**Fig. 5** Identification of the sulfate ester by sulfatase digestion (**a**) and ion chromatography (**b**). **a** Reversed-phase HPLC of the arylsulfatase digest of BG360. *Top* BG360 incubated without enzyme, *middle* BG360 digested with keyhole limpet sulfatase, *bottom* 3Hyk. **b** Ion chromatography of acid hydrolysates and inorganic ions on an anion-exchange column (Mono-Q). Ions (about 100 nmol each) were detected by indirect UV detection at 285 nm. From *top to bottom*: chloride (NaCl), phosphate ($KH_2PO_4$), sulfate ($(NH_4)_2SO_4$), acid hydrolysate of Tyr($SO_3H$), acid hydrolysate of BG360. The *vertical arrow* indicates 0.01 absorbance unit (AU)

coagulation factors by 3Hyk($SO_3H$) at around 1 mM concentration was observed.

## Discussion

3Hyk($SO_3H$) in biological materials was first reported in 1952 from the urine of pyridoxine-deficient rat fed with tryptophan (Dalgliesh 1952), and later it was chemically synthesized by persulfate oxidation of Kyn (Boyland et al. 1956). A few sporadic studies had followed these reports until 1975 (Moon and Morris 1975) regarding the tryptophan metabolism, but there was practically none thereafter. This molecule in vertebrates has been recognized as a breakdown product of tryptophan to be excreted into urine, and, therefore, the present report seems to be the first description of its unambiguous presence in the normal vertebrate tissue. In 1963, 3Hyk($SO_3H$) was isolated from whole body extracts of a blood-sucking insect, *Rhodnius prolixus*, as a novel kynurenine derivative (Viscontini and Schmid 1963), which was termed "rhodnitin," and then in 1967 from *Triatoma infestans*, also a blood-sucking insect of South America (Shimamune et al. 1967). These studies focused on tryptophan metabolites in insect development and coloration, and its possible relation to blood-sucking or parasitism was not considered. Since both lampreys and these insects are parasitic blood-feeders, it is tempting to speculate a role for 3Hyk($SO_3H$) in blood-feeding and parasitism despite the absence of direct inhibition of thrombin and factor Xa activity. In addition to the direct inhibitors of coagulation factors, invertebrate blood feeders secrete a wide array of pharmacologically active substances including nitric oxide to facilitate blood-sucking (Ribeiro and Walker 1994; Yuda et al. 1997; Golodne et al. 2003; Andersen 2010). 3Hyk is not merely a transient intermediate in the tryptophan catabolism, but it has profound biochemical, neurological, and pharmacological activities. It is a paralytic agent (Chiou et al. 1998), a cytotoxin to human neurons and glial cells (Chiou et al. 1998), a covalent inhibitor of rat brain creatine kinase (Cornelio et al. 2006), and an antimicrobial agent (Narui et al. 2009). Consequently, the present sulfonated derivative of 3Hyk may well be a similar bioactive molecule. Although sulfoconjugation of xenobiotics is established as a major detoxification mechanism, many endogenous small molecules, such as steroids, catecholamines, thyroid hormones, and various peptide hormones, also undergo sulfonation (Strott 2002). This is highly suggestive of biological importance of the modification, by which activities of the parent molecules are modulated and novel ones are brought about. In this respect, it is noteworthy that larvae of sea lampreys (*Petromyzon marinus*) secrete several sulfonated bile acids as migratory pheromone for adults (Li et al. 1995), and adult male lampreys release 3-ketopetromyzonol sulfate as mating sex pheromone for females (Li et al. 2002). Since buccal glands are present in some nonparasitic lamprey species (Potter et al. 1995) their function is certainly not restricted to blood-feeding or parasitism, and 3Hyk($SO_3H$), if also present in them, might serve a much broader physiological role. Exploring

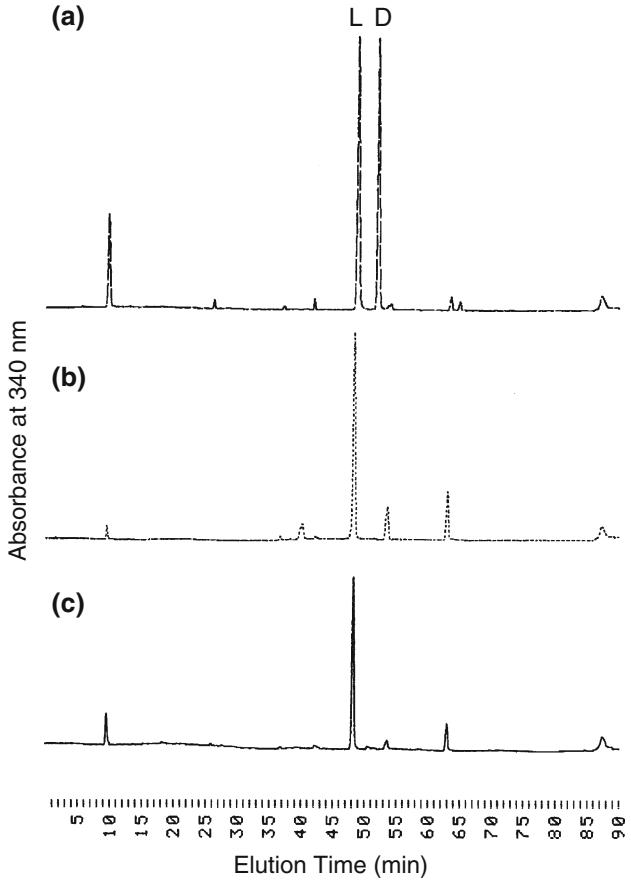


**Fig. 6** Analysis of the enantiomeric configuration of 3Hyk moiety in BG360 by Marfey's reagent. **a** Authentic D,L-3Hyk. **b** L-3Hyk prepared by acetylation of D,L-3Hyk followed by acylase digestion. **c** Acid-hydrolyzed BG360

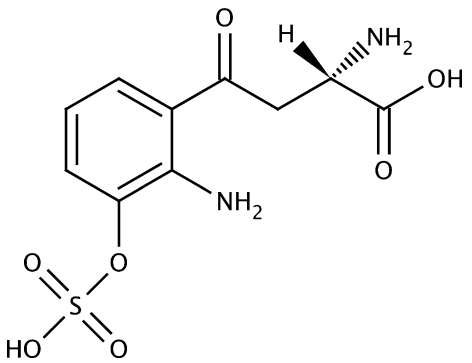


**Fig. 7** Structure of L-3-hydroxykynurenine-*O*-sulfate

biological activity and pharmacological potential of this long forgotten molecule is therefore considered particularly promising.

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**Conflict of interest** The authors declare that they have no conflict of interest.

## References

Andersen JF (2010) Structure and mechanism in salivary proteins from blood-feeding arthropods. Toxicon 56:1120–1129

Atherton SJ, Dillon J, Gaillard ER (1993) A pulse radiolysis study of the reactions of 3-hydroxykynurenine and kynurenine with oxidizing and reducing radicals. Biochim Biophys Acta 1158:75–82

Boyland E, Sims P, Williams DC (1956) The oxidation of tryptophan and some related compounds with persulphate. Biochem J 62:546–550

Chiou SJ, Kotanen S, Cerstiaens A et al (1998) Purification of toxic compounds from larvae of the gray fleshfly: the identification of paralysins. Biochem Biophys Res Commun 246:457–462

Cornelio AR, Rodrigues V Jr, de Souza Wyse AT, Dutra-Filho CS, Wajner M, Wannmacher CM (2006) Inhibition of creatine kinase activity from rat cerebral cortex by 3-hydroxykynurenine. Brain Res 1124:188–196

Corral-Rodríguez MA, Macedo-Ribeiro S, Pereira PJ, Fuentes-Prior P (2010) Leech-derived thrombin inhibitors: from structures to mechanisms to clinical applications. J Med Chem 53:3847–3861

Dalgliesh CE (1952) The relation between pyridoxin and tryptophan metabolism, studied in the rat. Biochem J 52:3–14

Denkert M, Hackzell L, Shill G, Sjorgen E (1981) Reversed-phase ion pair chromatography with UV-absorbing ions in the mobile phase. J Chromatogr 218:31–34

Fox JW, Serrano SMT (2007) Approaching the golden age of natural product pharmaceuticals from venom libraries: an overview of toxins and toxin-derivatives currently involved in therapeutic or diagnostic applications. Curr Pharm Des 13:2927–2934

Gage SH, Gage-Day M (1927) The anti-coagulating action of the secretion of the buccal glands of the lampreys (Petromyzon, Lampetra and Entosphenus). Science 66:282–284

Gess RW, Coates MI, Rubidge BS (2006) A lamprey from the Devonian period of South Africa. Nature 443:981–984

Golodne DM, Monteiro RQ, Graca-Souza AV, Silva-Neto MA, Atella GC (2003) Lysophosphatidylcholine acts as an anti-hemostatic molecule in the saliva of the blood-sucking bug *Rhodnius prolixus*. J Biol Chem 278:27766–27771

Heisz O (1983) Ionen-Chromatographie durch HPLC und indirekte UV-Detektion. GIT Fachz Lab 27:596–600

Hopla CE, Durden LA, Keirans JE (1994) Ectoparasites and classification. Rev Sci tech Off Int Epiz 13:985–1017

Ito N, Mita M, Takahashi Y, Matsushima A, Watanabe YG, Hirano S, Odani S (2007) Novel cysteine-rich secretory protein in the buccal gland secretion of the parasitic lamprey, *Lethenteron japonicum*. Biochem Biophys Res Commun 58:35–40

Kido R, Noguchi T, Matsumura Y (1967) The isolation and identification of 5-hydroxy-L-kynurenine from the urine of hens. Biochem Biophys Acta 141:270–275

Lehane M (2005) The biology of blood-sucking in insects, 2nd edn. Cambridge University Press, Cambridge

Li W, Sorensen PW, Gallaher DD (1995) The olfactory system of migratory adult sea lamprey (*Petromyzon marinus*) is specifically and acutely sensitive to unique bile acids released by conspecific larvae. J Gen Physiol 105:569–587

Li W, Scott AP, Siefkes MJ, Yan H, Liu Q, Yun SS, Gage DA (2002) Bile acid secreted by male sea lamprey that acts as a sex pheromone. Science 296:138–141

Marfey P (1984) Determination of D-amino acids. II. Use of a bifunctional reagent, 1,5-difluoro-2,4-dinitrobenzene. Carlsberg Res Commun 49:591–596

Moon RJ, Morris KM (1975) Quantitative analysis of tryptophan metabolites in urine of normal and endotoxin poisoned mice. Acta Vitaminol Enzymol 29:48–51

Narui K, Noguchi N, Saito A, Kakimi K, Motomura N, Kubo K, Takamoto S, Sasatsu M (2009) Anti-infectious activity of tryptophan metabolites in the L-tryptophan-L-kynurenine pathway. Biol Pharm Bull 32:41–44

Potter IC, Thomson GJ, Cook RD, Cox JM, Macey DJ (1995) Buccal glands of adults of the lamprey *Mordacia mordax*, including comparisons with other species. J Morphol 226:339–349

Rawlings ND, Barrett AJ, Bateman A (2010) MEROPS: the peptidase database. Nucleic Acids Res 38:D227–D233

Ribeiro JM, Walker FA (1994) High affinity histamine-binding and antihistaminic activity of the salivary nitric oxide-carrying heme protein (nitrophorin) of *Rhodnius prolixus*. J Exp Med 180:2251–2257

Scaloni A, Simmaco A, Bossa F (2003) D-Amino acid analysis. Methods Mol Biol 211:169–180

Shimamune A, Ricardo A, Naquira C (1967) Fluorescing substances in the blood-sucking bug, *Triatoma infestans* I. Seikagaku 39:29–45

Strott CA (2002) Sulfonation and molecular action. Endocr Rev 23:703–732

Tallan HH, Bella ST, Stein WH, Moore S (1955) Tyrosine-*O*-sulfate as a constituent of normal human urine. J Biol Chem 217:703–708

Vetter I, Davis JL, Rash LD, Anangi R, Mobli M, Alewood PF, Lewis RJ, King GF (2010) Venomics: a new paradigm for natural products-based drug discovery. Amino Acids. doi:10.1007/s00726-010-0516-4

Viscontini M, Schmid H (1963) Fluoreszierende Stoffe aus *Rhodnius prolixus* Stål (Hemiptera, Heteroptera) Ein neues Kynureninderivat: 3-hydroxykynurenin-schwefelsäure ester = Rhodnitin. Helvetica Chim Acta 46:2509–2516

Xiao R, Li QW, Perrett S, He RQ (2007) Characterisation of the fibrinogenolytic properties of the buccal gland secretion from *Lampetra japonica*. Biochimie 89:383–392

Yamazaki Y, Morita T (2007) Snake venom components affecting blood coagulation and the vascular system: structural similarities and marked diversity. Curr Pharm Des 13:2872–2886

Yuda M, Higuchi K, Sun J, Kureishi Y, Ito M, Chinzei Y (1997) Expression, reconstitution and characterization of prolixin-S as a vasodilator—a salivary gland nitric-oxide-binding hemoprotein of *Rhodnius prolixus*. Eur J Biochem 249:337–342