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D-Aspartate oxidase and free acidic D-amino acids in lower vertebrates

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Abstract

D-Aspartate oxidase and free D-aspartate and/or D-glutamate have been found in various tissues of lower vertebrates including avians, amphibians and fishes. The enzymes of those animals were similar to the mammalian enzymes in substrate and inhibitor specificity. *N*-Methyl-D-aspartate is the best substrate of the enzymes of those animals, followed by D-aspartate, *meso*-2,3-diaminosuccinate and D-glutamate. In avians and amphibians, the highest activity was found in the kidneys, whereas, in fishes, hepatic activity was equal to or greater than renal activity. Male chickens had significantly higher activities in the liver, kidneys and pancreas than female. However, no significant sex difference was observed in other animals.

Substantial amounts of D-aspartate and/or D-glutamate were detected in all tissues examined, irrespective of species. With the exception of the spleen or testes of some species, the D-glutamate content was equal to or greater than the D-aspartate content. The amounts of D-enantiomer present were significantly different between two sexes in several tissues of avians and amphibians. However, no common relationship was observed between the D-enantiomer contents and D-aspartate oxidase activity. The involvement of D-aspartate in the control of androgen secretion by the ovary was demonstrated in a green frog.

Keywords: D-Aspartate oxidase; D-Aspartate; D-Glutamate; Distribution; Avians; Amphibians; Fishes

1. Introduction

D-Aspartate oxidase is a FAD enzyme that catalyzes the oxidative deamination of dicarboxylic D-amino acids such as D-aspartate and D-glutamate, as well as their derivatives [1]. Activity catalyzing the oxidation of these D-amino acids was first discovered in rabbit kidney and liver by Still et al. in 1949 [2]. It was not until 1987 that D-aspartate oxidase was first purified to homogeneity from bovine kidney [3], while D-aspartate oxidase activity has been found in many mammalian tissues including human [1]. This enzyme has also been found in cephalopods [4], crustaceans [5], fishes [6], amphibians [7], avians [8] and microorganisms [9,10], and has been purified from octopus (*Octopus vulgaris*) [11,12] and the yeast *Cryptococcus humicolus* UJ1 [13]. Further, D-glutamate oxidase, which prefers D-glutamate to D-aspartate as a

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substrate, has been purified from the yeast *Candida boidinii* 2201 [14]. The peroxisomal localization of this enzyme has been demonstrated in the kidney cortex of rat, bovine and sheep [15], the liver of rat and human [16], and the yeast *C. humicolus* UJ1 [17].

As for the probable *in vivo* substrate of D-aspartate oxidase, free D-aspartate has been found in a gastropod [18], marine bivalves [19,20], cephalopods [21–24], crustaceans [25,26], tunicates [27], fishes [6], amphibians [7,28], avians [8,29,30] and mammals including human [31,32]. Free D-glutamate has been found in insects [33], crustaceans [25,26], fishes [6], avians [8] and mammals [34]. The present review describes the distribution of D-aspartate oxidase and free acidic D-amino acids in various tissues of lower vertebrates, in comparison with that reported in mammals.

2. D-Aspartate oxidase

2.1. Enzyme properties

D-Aspartate oxidase activity has been found in various tissues of fishes, amphibians and avians [6–8]. D-Aspartate oxidase activity required native enzyme, oxygen and substrate. FAD was required for activity to different extents depending on the species from which enzyme was obtained. This is probably due to the difference in the ratio of holoenzyme to apoenzyme, which depends on the tissue content of FAD, affinity of the apoenzyme for the coenzyme, or other factors. In addition, FMN could not replace FAD.

The effects of selective inhibitors on D-aspartate oxidase activity were compared in tissue homogenates of various species (Table 1). *meso*-Tartrate, a strong competitive inhibitor of mammalian D-aspartate oxidase [1], inhibited the oxidation of D-aspartate, while having no significant effect on the oxidation of D-alanine, in all species. Conversely, benzoate, a competitive inhibitor of D-amino acid oxidase [1], inhibited the oxidation of D-alanine more strongly than that of D-aspartate, although benzoate significantly inhibited the oxidation of D-aspartate by the carp and crucian carp enzymes. The selective inhibition of D-aspartate oxidase and D-amino acid oxidase, respectively, by *meso*-tartrate and benzoate is in good agreement with data obtained from mammalian enzymes [35,36].

The apparent substrate specificity of D-aspartate oxidase from tissue homogenates of various species is given in Table 2. The oxidation rates of D-glutamate and *meso*-2,3-diaminosuccinate were considerably slower than that of D-aspartate, and *meso*-2,3-diaminosuccinate was a slightly better substrate than D-glutamate for the enzymes of some animals. The low preference of D-aspartate oxidase for D-glutamate and *meso*-2,3-diaminosuccinate was also reported for mammalian D-aspartate oxidase [15,16]. Of parti-

Table 1
Effects of inhibitors on D-aspartate oxidase and D-amino acid oxidase activities from tissues of lower vertebrates[a]

Species [references]	Tissue	Relative activity (%)			
		D-Aspartate oxidase		D-Amino acid oxidase	
		meso-Tartrate	Benzoate	*meso*-Tartrate	Benzoate
Chicken [8]	Kidney	39	89	96	4
Pigeon [8]	Kidney	63	94	98	43
Clawed toad [7]	Liver	49	98	89	59
Japanese newt [7]	Liver	33	91	84	42
Carp [6]	Liver	79 (47)	57	96 (89)	7
Crucian carp [6]	Liver	99 (44)	69	98 (96)	9
Rainbow trout [6]	Liver	66	97	99	67
Yellowtail [6]	Liver	30	97	94	22
Sea bream [6]	Liver	66	95	96	39

[a] D-Aspartate oxidase and D-amino acid oxidase activities were assayed with 20 mM D-aspartate and 20 mM D-alanine, respectively as a substrate. The final concentration of inhibitors in the assay mixture was 10 mM. Relative activity with 40 mM inhibitors are shown in parentheses.

Table 2
Substrate specificity of D-aspartate oxidase from tissues of lower vertebrates[a]

Species [references]	Tissue	Relative activity (%)			
		D-Aspartate	D-Glutamate	*N*-Methyl-D-aspartate	*meso*-2,3-Diaminosuccinate
Chicken [8]	Kidney	100	23	186	37
Pigeon [8]	Kidney	100	21	103	21
Clawed toad [7]	Liver	100	15	194	–
Japanese newt [7]	Liver	100	33	202	–
Carp [6]	Liver	100	5	188	46
Crucian carp [6]	Liver	100	7	176	26
Rainbow trout [6]	Liver	100	11	151	19
Yellowtail [6]	Liver	100	28	102	26
Sea bream [6]	Liver	100	3	204	0

[a] The final concentration of substrates was 20 mM. –, data not available.

cular interest is the fact that the oxidation rate of *N*-methyl-D-aspartate (NMDA) is equal to or higher than that of D-aspartate in the lower vertebrates. This trait seems to be shared by the enzymes of mammals [12,15,16,37,38], although the opposite relation is reported for human liver [16], rat kidney cortex [15] and recombinant human brain D-aspartate oxidase [39]. On the contrary, a lower preference for NMDA than for D-aspartate was reported for D-aspartate oxidase from octopus [12] and microorganisms, such as the fungus *Fusarium sacchari* [10], the yeast *C. humicolus* [13] and the yeast *C. boidinii* 2201 [14].

NMDA is a potent agonist of one of the subtypes of glutamate receptors in higher animals [40]. Although NMDA was regarded as a solely artificial compound, it was recently crystallized from a huge amount of *Scapharca broughtonii* foot muscle [41]. More recently, we determined the NMDA contents in various tissues of *S. broughtonii* and *S. subcrenata*, through a newly-developed method of high-performance liquid chromatography [42]. In addition, we could not find any D-aspartate oxidase activity in *S. broughtonii* tissues [20].

2.2. Enzyme distribution

The distribution of D-aspartate oxidase in given tissues of various species is compared in Table 3. In avians and amphibians, the specific activity is comparable to that in the corresponding tissues of rodents [16,36,43], and the tissue distribution profile of the enzyme is similar to that reported in mammals: renal activity is usually several-fold higher than hepatic activity. In contrast, in fishes the activity in the liver was greater than or equal to that in the kidney, suggesting a marked species difference.

A sex difference in activity was observed in chickens; the hepatic, renal and pancreatic activities were significantly higher in male than female. The hormonal factors affecting enzyme activity appear to be different between chicken and rodents, since the hepatic and/or renal activity of rats and mice is, in contrast, higher in female than male [44,45]. Moreover, the hepatic activity in pregnant female rats was higher than the control females and decreased immediately after parturition [44]. Further studies are required to identify the hormonal factors affecting the enzyme activity in lower vertebrates, as well as in mammals.

In addition to hormonal factors, the administration of D-aspartate to mice increased hepatic activity, irrespective of sex [45,46]. An interesting question is whether the administration of D-aspartate also induces enzyme activity in lower vertebrates.

3. Free acidic D-amino acids

Among tissues of lower vertebrates, chicken egg (embryo) was the first one that was found to contain D-aspartate [29]. The total D-aspartate content of the eggs increased four-fold during incubation for 12 days, showing that the D-amino acid was released from a bound form or synthesized *de novo*. Subsequently, free D-aspartate and D-glutamate were found in many tissues of various lower vertebrates (Table 4). The tissue

Table 3
Specific activity of D-aspartate oxidase in tissues of lower vertebrates[a]

Species [references] (age or body weight)	Tissue	Specific activity (nmol/min per mg protein)		
		Male	Mixed-sex[b]	Female
Chicken [8] (2.5 months)	Liver	0.57 ± 0.12[c]		0.32 ± 0.10
	Kidney	2.94 ± 0.21[c]		2.22 ± 0.61
	Brain	0.33 ± 0.03		0.29 ± 0.06
	Heart	0.46 ± 0.23		0.36 ± 0.19
	Pancreas	0.34 ± 0.15[c]		0.18 ± 0.07
	Spleen	0.06 ± 0.02		0.07 ± 0.02
	Adrenal gland	0.20 ± 0.12		0.22 ± 0.08
	Testis	0.23 ± 0.04		
	Ovary			0.30 ± 0.10
Pigeon [8] (8 months)	Liver	0.73 ± 0.16		0.55 ± 0.10
	Kidney	5.32 ± 1.64		4.66 ± 1.28
	Brain	0.08 ± 0.08		0.08 ± 0.03
	Heart	0.20 ± 0.09		0.14 ± 0.07
	Pancreas	0.25 ± 0.19		0.35 ± 0.19
	Spleen	0.03 ± 0.03		0.03 ± 0.01
	Testis	0.06 ± 0.03		
	Ovary			0.57 ± 0.12
Clawed toad [7] (adults)	Liver	0.48 ± 0.07		0.67 ± 0.31
	Kidney	2.65 ± 0.69		3.07 ± 0.90
	Brain	0.08[d]		0.09[d]
Japanese newt [7] (adults)	Liver	0.80 ± 0.27		0.74 ± 0.39
	Kidney	1.80 ± 0.41		1.60 ± 0.25
	Brain	0.03[d]		0.03[d]
Carp [6] (0.9–1.2 kg)	Liver		0.48 ± 0.33	
	Kidney		0.76 ± 0.38	
	Brain		0.07 ± 0.03	
Crucian carp [6] (100–140 g)	Liver		1.19 ± 0.21	
	Kidney		0.60 ± 0.13	
	Brain		0.09 ± 0.02	
Rainbow trout [6] (90–130 g)	Liver		2.33 ± 0.38	
	Kidney		0.21 ± 0.09	
	Brain		0.12 ± 0.05	
Yellowtail [6] (2.2–2.5 kg)	Liver		0.55 ± 0.47	
	Kidney		0.62 ± 0.12	
	Brain		0.53 ± 0.16	
Sea bream [6] (1.1–1.3 kg)	Liver		1.44 ± 0.54	
	Kidney		1.19 ± 0.68	
	Brain		0.04 ± 0.04	

[a] Activity is measured with tissue homogenates and expressed as means ± S.D.
[b] The data are of mixed-sex, because of no clear difference between two sexes.
[c] Statistical significance ($P < 0.05$) as compared to corresponding female tissue.
[d] The activity of one pooled brain sample from 6 to 10 animals is shown.

contents of D-aspartate and/or D-glutamate were of the same order of magnitude as those reported in mammalian tissues [31,32,34]. As observed in adult rat tissues [34], the D-glutamate content was greater than or equal to the D-aspartate content in most tissues except the spleen of chickens and some fishes, and the testis of sea bream. This data appears to be consistent with the substrate specificity of D-aspartate oxidase (Table 2).

The contents of D-aspartate and/or D-glutamate were significantly different between the sexes in chicken

Table 4
Contents and percentages of free D-aspartate and D-glutamate in tissues of lower vertebrates[a]

Species [references] (age/body weight)	Tissue	Content (nmol/g) and $D/D+L$ (% in parentheses)					
		D-Aspartate			D-Glutamate		
		Male	Mixed-sex[b]	Female	Male	Mixed-sex[b]	Female
Chicken [29]	Egg (incubation day 1)		44.5 ± 9.2				
			(0.16 ± 0.03)				
	Egg (incubation day 12)		159 ± 30				
			(0.6 ± 0.1)				
Chicken [30] (1.5 kg, control)	Muscle		40 ± 10				
	Liver		70 ± 20				
	Kidney		100 ± 40				
	Brain		80 ± 10				
Chicken [8] (2.5 months)	Liver	63.7 ± 20.2		65.4 ± 18.3	55.4 ± 19.2		69.7 ± 41.4
		(1.86 ± 0.64)		(1.94 ± 0.55)	(1.00 ± 0.37)		(1.02 ± 0.36)
	Kidney	42.9 ± 8.8		37.8 ± 8.8	58.3 ± 9.4[c]		34.9 ± 9.0
		(3.26 ± 0.37)		(3.01 ± 0.48)	(0.79 ± 0.09)[c]		(0.47 ± 0.09)
	Brain	33.8 ± 3.0		36.4 ± 9.3	38.5 ± 5.1		52.6 ± 33.4
		(1.43 ± 0.07)		(1.51 ± 0.10)	(0.54 ± 0.08)		(0.64 ± 0.28)
	Heart	37.8 ± 6.8[c]		56.4 ± 11.9	76.5 ± 8.9[c]		93.5 ± 8.7
		(0.96 ± 0.03)		(1.09 ± 0.17)	(0.74 ± 0.02)[c]		(0.80 ± 0.05)
	Pancreas	10.2 ± 1.3[c]		6.3 ± 0.9	75.2 ± 46.8[c]		22.6 ± 11.5
		(4.83 ± 1.73)		(3.19 ± 0.92)	(2.05 ± 0.91)[c]		(0.85 ± 0.46)
	Spleen	131.2 ± 28.2		103.4 ± 58.0	51.6 ± 43.1		30.6 ± 12.0
		(3.30 ± 0.43)		(3.00 ± 0.31)	(0.59 ± 0.40)		(0.52 ± 0.17)
	Adrenal gland	23.6 ± 11.4		30.2 ± 17.1	32.8 ± 20.0		30.8 ± 13.1
		(3.45 ± 0.16)		(2.84 ± 0.04)	(1.14 ± 0.13)		(0.87 ± 0.12)
	Testis[d]	9.4 ± 5.0			20.1 ± 9.7		
		(1.72 ± 0.51)			(0.47 ± 0.20)		
Pigeon [8] (8 months)	Liver	10.5 ± 4.7[c]		24.3 ± 4.6	12.1 ± 8.2[c]		64.9 ± 14.3
		(1.16 ± 0.11)		(1.26 ± 0.26)	(0.41 ± 0.17)[c]		(1.08 ± 0.09)
	Kidney	43.0 ± 6.8[c]		53.8 ± 8.7	94.4 ± 12.9[c]		135.7 ± 34.7
		(1.58 ± 0.18)[c]		(1.94 ± 0.09)	(0.94 ± 0.08)[c]		(1.12 ± 0.12)
	Brain	25.1 ± 2.0		27.1 ± 3.9	56.4 ± 3.5		52.4 ± 3.2
		(1.21 ± 0.18)		(1.29 ± 0.03)	(0.77 ± 0.04)		(0.77 ± 0.02)
	Heart	41.2 ± 14.3		42.3 ± 15.8	79.4 ± 24.7		67.0 ± 10.3
		(1.69 ± 0.54)		(1.50 ± 0.28)	(1.43 ± 0.43)		(1.29 ± 0.14)
	Pancreas	17.7 ± 10.0		14.9 ± 7.2	75.4 ± 44.9		54.9 ± 36.4
		(2.43 ± 1.19)		(2.07 ± 0.73)	(1.41 ± 0.91)		(1.08 ± 0.46)
	Testis[d]	9.6 ± 2.3			30.4 ± 24.5		
		(2.42 ± 0.91)			(0.67 ± 0.38)		
Clawed toad [7] (adults)	Liver	11.9 ± 3.8		14.8 ± 4.3			
		(2.0 ± 0.8)		(1.5 ± 0.8)			
	Kidney	3.3 ± 0.9		2.9 ± 0.7			
		(0.6 ± 0.1)		(0.6 ± 0.1)			
Japanese newt [7] (adults)	Liver	9.3 ± 3.5[c]		21.8 ± 16.1			
		(4.1 ± 1.2)		(3.3 ± 2.5)			
	Kidney	11.0 ± 2.9[c]		6.8 ± 1.5			
		(2.3 ± 1.2)		(1.8 ± 0.8)			
Green frog [28]	Ovary (March)			2.5 ± 1.1			
	Ovary (October)			58.0 ± 10.1			

Table 4 (*Continued*)

Species [references] (age/body weight)	Tissue	Content (nmol/g) and $D/D+L$ (% in parentheses)					
		D-Aspartate			D-Glutamate		
		Male	Mixed-sex[b]	Female	Male	Mixed-sex[b]	Female
Carp [6] (0.9–1.2 kg)	Liver		41.2 ± 28.3 (5.1 ± 4.3)			40.3 ± 10.3 (1.3 ± 0.3)	
	Kidney		37.1 ± 8.1 (4.8 ± 1.9)			23.5 ± 8.0 (1.0 ± 0.5)	
	Spleen		74.5 ± 15.3 (9.1 ± 4.8)			16.0 ± 4.3 (0.7 ± 0.2)	
	Testis	16.4, 17.9 (5.5, 1.9)			31.8, 33.2 (0.6, 0.6)		
	Ovary			19.0 ± 11.9 (2.3 ± 0.3)			32.7 ± 22.6 (0.7 ± 0.1)
Crucian carp [6] (100–140 g)	Liver		21.6 ± 7.6 (1.6 ± 0.6)			53.0 ± 20.9 (1.1 ± 0.3)	
	Kidney		27.7 ± 6.7 (3.8 ± 0.9)			44.5 ± 25.7 (1.5 ± 0.6)	
	Brain		5.5 ± 1.2 (5.0 ± 4.1)			36.4 ± 5.0 (0.9 ± 0.1)	
	Testis	23.9 ± 13.6 (1.3 ± 0.4)			23.5 ± 16.3 (1.1 ± 0.7)		
	Ovary			3.5, 4.3 (3.5, 4.3)			1.5, 1.1 (0.8,0.5)
Rainbow trout [6] (90–130 g)	Liver		15.7 ± 5.9 (3.3 ± 2.5)			31.7 ± 6.9 (1.0 ± 0.3)	
	Kidney		30.5 ± 12.5 (5.8 ± 4.7)			31.4 ± 8.8 (0.9 ± 0.2)	
	Brain		8.8 ± 3.5 (1.7 ± 0.9)			27.4 ± 9.7 (0.8 ± 0.2)	
	Spleen		34.4 ± 14.0 (3.7 ± 1.6)			22.0 ± 7.0 (1.0 ± 0.4)	
	Heart		4.3 ± 1.7 (3.4 ± 0.5)			10.8 ± 6.8 (1.1 ± 0.6)	
	Muscle		1.8 ± 0.1 (5.2 ± 1.7)			3.2 ± 1.7 (2.3 ± 1.1)	
	Testis	2.5 (13.7)			14.5 (2.9)		
	Ovary			3.4, 3.9 (3.1, 1.6)			15.3, 10.0 (1.3, 0.7)
Yellowtail [6] (2.2–2.5 kg)	Liver		30.9 ± 36.8 (9.3 ± 8.7)			453 ± 440 (4.2 ± 4.0)	
	Kidney		17.2 ± 6.2 (2.7 ± 0.5)			41.8 ± 41.9 (0.9 ± 0.5)	
	Brain		14.8 ± 4.7 (1.4 ± 0.1)			45.3 ± 7.5 (1.2 ± 0.1)	
	Spleen		25.9 ± 13.9 (1.7 ± 0.1)			18.8 ± 11.8 (0.9 ± 0.3)	
	Heart		17.4 ± 1.7 (2.2 ± 0.6)			25.5 ± 0.9 (2.0 ± 0.4)	
	Muscle		2.6 ± 1.0 (7.3 ± 2.7)			9.4 ± 7.3 (2.5 ± 0.6)	
	Testis[d]	1.1 (4.9)			0.3 (0.4)		
	Ovary			11.3, 19.7 (1.2, 1.6)			11.2, 40.3 (0.9, 1.1)

Table 4 (*Continued*)

Species [references] (age/body weight)	Tissue	Content (nmol/g) and $D/D+L$ (% in parentheses)					
		D-Aspartate			D-Glutamate		
		Male	Mixed-sex[b]	Female	Male	Mixed-sex[b]	Female
Sea bream [6] (1.1–1.3 kg)	Liver		20.0 ± 5.7 (6.5 ± 1.9)			176 ± 129 (1.5 ± 0.9)	
	Kidney		44.1 ± 33.9 (4.5 ± 1.5)			74.6 ± 29.1 (2.2 ± 1.4)	
	Brain		16.9 ± 7.0 (3.5 ± 2.1)			59.5 ± 7.5 (1.3 ± 0.4)	
	Spleen		45.0 ± 22.6 (4.2 ± 2.4)			76.6 ± 30.8 (2.3 ± 1.0)	
	Heart		32.0 ± 2.9 (3.1 ± 0.6)			85.5 ± 57.0 (3.6 ± 3.4)	
	Muscle		8.4 ± 2.0 (11.7 ± 5.4)			25.6 ± 9.0 (2.1 ± 1.1)	
	Testis	217, 230 (3.5, 2.6)			104, 56.2 (2.5, 1.1)		
	Ovary			32.3, 18.6 (4.8, 6.0)			164, 41.5 (10.9, 1.7)

[a] The contents are expressed as means ± S.D., when the number of samples is three or more.
[b] The data are of mixed-sex, because of no indication of sex or no clear difference between two sexes.
[c] Statistical significance ($P < 0.05$) as compared to corresponding female tissue.
[d] Unpublished data.

kidney, heart and pancreas, pigeon liver and kidney and Japanese newt liver and kidney. However, no common relationship suggesting the function of the enzyme was seen between the amino acid contents and enzyme activity in those animal tissues (Tables 3 and 4). This is in marked contrast to the sex-dependent inverse relationship between the contents of acidic D-amino acids and enzyme activity observed in rat liver and kidney [34] and mouse liver [45].

D-Aspartate contents appeared higher in the testis than ovary in the sea bream and crucian carp, although the small number of samples might have skewed interpretation. In a green frog, *Rana esculenta*, the ovarian content of D-aspartate was found to be low in spawning females in March and was high in October, and testosterone levels in plasma and ovary were found to be inversely related to D-aspartate contents [28]. Further, the administration of D-aspartate decreased plasma testosterone levels while that of D-glutamate or D-alanine was ineffective. These findings suggest that D-aspartate is involved in the control of androgen secretion by the ovary. Recently, it was reported that D-aspartate stimulates testosterone synthesis in rat testis [47] and rat Leydig cells [48,49]. These apparently inconsistent results might be explained by the difference in reproductive organs and species. Further studies are required to obtain conclusive evidence.

4. Discussion

This review has described the distribution of D-aspartate oxidase and free acidic D-amino acids in lower vertebrates. The inhibitor and substrate specificity of the enzymes studied are similar to those of mammalian enzymes. Of particular interest is that NMDA is equally good or superior to D-aspartate as a substrate for the enzymes of various vertebrates including mammals. A high-performance liquid chromatographic method is now available to determine minute concentrations of NMDA in small amounts of biological sample [42]. If NMDA is found to be present in vertebrate tissues in future, it is possible that NMDA functions as another physiological substrate for D-aspartate oxidase in those vertebrates.

The various tissues of lower vertebrates contained both D-aspartate oxidase and free acidic D-amino acids, at a level comparable to that found in mammalian tissues. This suggests that these amino acids are in vivo substrates of D-aspartate oxidase in these animals. Some tissues showed significant differences between the sexes in D-aspartate oxidase activity or D-aspartate and/or D-glutamate content, although no common relationship between enzyme activity and D-amino acid content was found. This suggests that the tissue levels of these D-enantiomers are also affected by several factors other than D-aspartate oxidase, such as amino acid transport systems and dietary conditions.

Many studies with rats suggest that D-aspartate is probably involved in the developmental processes of organs [32] and hormonal regulation in endocrine tissues, such as the pineal gland [50] and testes [47–49]. It will be interesting to discover whether the suggested role of D-aspartate in mammals is also played in lower vertebrates. Further studies are required to examine this possibility, although D-aspartate is reported to be involved in the control of androgen secretion by the ovary in a green frog, *R. esculenta*.

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