

雌雄文昌鱼同工酶的表型差异*

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摘要 本文应用聚丙烯酰胺凝胶电泳结合生化染色方法分析了雌雄文昌鱼中苹果酸酶、苹果酸脱氢酶、酸性磷酸酶和酯酶四种同工酶的酶谱。首次发现苹果酸酶、苹果酸脱氢酶和酸性磷酸酶表型在文昌鱼雌性和雄性个体之间存在差异, 而在同一性别不同个体之间无差异。酯酶表型较复杂, 不但在不同性别个体之间而且在同一性别不同个体之间都出现一定差异 [动物学报 50 (1): 62-67, 2004]。

关键词 文昌鱼 苹果酸酶 苹果酸脱氢酶 酸性磷酸酶 酯酶

Sex-related differences in isozymic phenotypes in amphioxus *Branchiostoma belcheri tsingtauense*

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Abstract Four isozymes, i. e. malic enzyme, malate dehydrogenase, acid phosphatase and esterase, in amphioxus *Branchiostoma belcheri tsingtauense*, were analyzed using polyacrylamide gel electrophoresis (PAGE) and biochemical staining. It was found that (1) the electrophoretic patterns of the isozymes showed sex-related differences between male and female amphioxus; (2) the electrophoretic patterns of malic enzyme, malate dehydrogenase and acid phosphatase did not show inter-individual difference in the same sex; (3) however, the electrophoretic patterns of esterase showed some differences in both sexes as well as in different individuals of the same sex [Acta Zoologica Sinica 50 (1): 62-67, 2004].

Key words Amphioxus, *Branchiostoma belcheri tsingtauense*, Malic enzyme, Malate dehydrogenase, Acid phosphatase, Esterase

同工酶的形成是生物在进化过程中对代谢适应的结果。同一种群中的不同个体、不同性别、同一个体的不同发育阶段以及同一个体不同组织、不同器官中同工酶的表达都存在差异。同工酶作为遗传、生化指标已广泛应用于分子遗传、种群遗传、系统进化、个体发育、杂交育种以及病理和毒理等方面的研究(熊全沫, 1992; Graf, 1989; Basaglia et al., 1990; Basaglia, 2002; Kobliakov et al., 1991; Alekhova et al., 2001)。

文昌鱼 (*Branchiostoma belcheri*) 是由无脊椎动物进化到脊椎动物的过渡型代表动物。自 1774 年 Pallas 首次发现文昌鱼以来, 有关其形态解剖、胚胎发育、系统进化和生理和生化等方面已有大量

研究报告 (Tung et al., 1958; Fang et al., 2003; 童第周等, 1964; 方永强等, 1999, 2001; 王勇等, 2001; 张士瑾等, 2001; 翁幼竹等 2001a, b)。相比之下, 对文昌鱼同工酶的研究十分有限 (Manwell, 1975; Schmidtke, 1977)。本文应用聚丙烯酰胺凝胶电泳和生化染色技术对文昌鱼 (*B. b. tsingtauense*) 雌、雄个体中苹果酸酶、苹果酸脱氢酶、酸性磷酸酶和酯酶四种酶的同工酶进行了比较研究。

1 材料与方法

1.1 生化试剂

-萘乙酸 (-naphthyl acetate)、固蓝 RR

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(Fast blue RR)、固酱紫 GBC (Garnet GBC)、L-苹果酸 (L-malic acid)、氧化型辅酶 (NADP)、吩嗪硫酸甲酯 (Phenazine methosulfate, PMS)、甲基噻唑四唑啉 (Methyl thiazolyl tetrazolium, MTT)、-萘磷酸 (β -naphthyl phosphate) 和固酱紫 GBC (Garnet GBC) 均购于 Sigma 公司, 其余试剂均为分析纯。

1.2 酶液制备

2002 年 7 月于青岛市沙子口采集性成熟文昌鱼, 雌、雄分开后置于 -70°C 冰箱保存备用。

文昌鱼个体很小 (成体平均体长 4 cm), 分离不同组织比较困难。本实验随机取雌、雄文昌鱼各 8-12 条, 逐条称重后, 按 1:3 (w/v) 比例加入 4 预冷的缓冲液。苹果酸酶、苹果酸脱氢酶、酸性磷酸酶和酯酶的提取分别按 Graf (1989)、Basaglia (2002)、Kubici et al. (1978) 以及 Robbi and Beaufay (1983) 方法。所用缓冲液分别为 25 mmol/L Tris-HCl (pH 7.4) 含 5 mmol/L $\text{MgCl}_2 + 5$ mmol/L KCl + 0.14 mol/L 蔗糖 + 0.5% Triton X-100 + 0.5% 巯基乙醇, 0.1 mmol/L Tris-HCl 缓冲液 (pH 7.0), 0.1 mol/L 乙酸缓冲液 (pH 5.0) 和 0.01 mol/L 磷酸盐缓冲液 (pH 7.4) 含 0.2% Triton X-100。文昌鱼先用眼科剪剪碎后, 于 0 匀浆。匀浆液于 4°C 离心后取上清液, 置于 -70°C 冰箱保存备用。

上清液蛋白质浓度测定按 Bradford (1976) 方法。所用参比蛋白为 1 mg/ml 牛血清白蛋白 (BSA)。

1.3 电泳和酶的染色

上清液和样品缓冲液 (pH 6.8 的 312 mmol/L

Tris-HCl + 50% 甘油 + 0.05% 溴酚蓝) 按 4:1 混合后加样。每个泳道加样品量为 20 μl , 总蛋白含量为 5 μg 。电泳采用高 pH 不连续系统 (250 mmol/L Tris + 200 mmol/L Gly, pH = 8.3)。苹果酸酶、苹果酸脱氢酶和酸性磷酸酶均采用 7.5% 聚丙烯酰胺凝胶电泳, 而酯酶采用 10% 聚丙烯酰胺凝胶电泳, 两者浓缩胶浓度都为 3%。电泳结束后, 将凝胶转入盛有新鲜染液的培养皿中, 按 Harris and Hopkinson (1976) 方法进行各种酶的染色。染色后, 取出凝胶, 用双蒸水冲洗两次, 转入 7% 醋酸或 7.5% 醋酸 - 30% 乙醇 - 15% 甘油的固定液中, 固定保存, 并用凝胶成像仪 (YLN2K) 记录结果。

1.4 命名

命名原则参照 Gosling (1982) 方法, 即以各种酶谱的相对迁移率 ($R_f = d/l$, d 为酶带迁移距离; l 为指示剂迁移距离) 由小到大依次命名。

2 结果

2.1 苹果酸酶 (Malic enzyme, ME, E. C. 1.

1. 1. 40)

所有 8 条雄性文昌鱼都只有一条酶带 ME-2, 而所有 8 条雌性文昌鱼除酶带 ME-2 之外, 还有染色较弱的 ME-1 带 (图 1, 表 1)。雌、雄文昌鱼之间 ME 的差异, 不仅表现在 ME 酶带的数量上, 而且表现在雌性文昌鱼 ME-2 酶带染色比雄性的酶带要深 (图 1)。显然, 苹果酸酶在同性别的文昌鱼个体间不存在差异, 而在雌、雄两性之间则有明显差异。

2.2 苹果酸脱氢酶 (Malate dehydrogenase, MDH, E. C. 1.

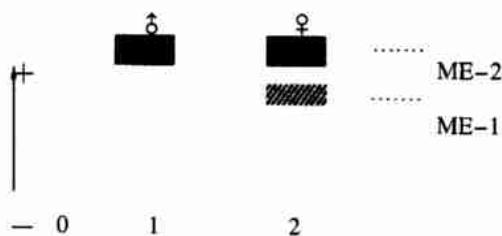
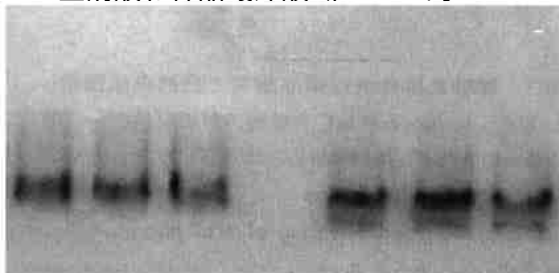


图 1 雌、雄文昌鱼苹果酸酶同工酶的电泳酶谱

0: 原点。1: 雄文昌鱼的苹果酸酶同工酶的电泳酶谱。2: 雌文昌鱼的苹果酸酶同工酶的电泳酶谱。ME-1: 苹果酸酶表达位点 1。ME-2: 苹果酸酶表达位点 2。

Fig. 1 Electrophoretogram of malic enzyme isozymes in male and female *Branchiostoma belcheri tsingtauense*

0: Origin). 1: The electrophoretogram of malic enzyme isozymes in male. 2: The electrophoretogram of malic enzyme isozymes in female. ME-1: Loci 1 expressed for malic enzyme. ME-2: Loci 2 expressed for malic enzyme.

1. 1. 37)

雌、雄文昌鱼都显示两条深染的酶带 MDH-2 和 MDH-4 和一条较弱的 MDH-1 带。但是，雌性文昌鱼除表达 MDH-1、MDH-2 和 MDH-4 三条酶带之外还表达一条较弱的酶带 MDH-3 (图 2, 表 1)。和 ME 酶一样, MDH 酶在雌性或雄性文昌鱼个体间谱带相同, 但在雌性和雄性之间, MDH 酶谱带却存在差异。

2. 3 酸性磷酸酶 (Acid phosphatase, ACP, E. C. 3. 1. 3. 2)

酸性磷酸酶在雌性和雄性文昌鱼中分别有 5 条和 4 条酶带。雌、雄文昌鱼都具有 ACP-4、ACP-5 和 ACP-6 三条酶带, 但雌性文昌鱼中还表达 ACP-

1 和 ACP-3 两条酶带, 而雄性文昌鱼则只显示 ACP-2。在所分析的 8 条雌性和 8 条雄性文昌鱼 ACP 酶谱中, 在同性个体内部均未发现差异, 而在雌、雄两性之间则存在带型差异 (图 3, 表 1)。

2. 4 酯酶 (Esterase, EST, E. C. 3. 1. 1. 1)

文昌鱼酯酶谱带比较复杂, 同一性别个体之间和不同性别个体之间谱带都有差异 (图 4, 表 2)。雌、雄文昌鱼都表达 EST-2 和 EST-11 两条酶带, 其中 EST-2 染色较强, 而 EST-11 染色很弱。另外, EST-1、EST-3 和 EST-5 酶带在大部分雄性个体中存在, 而 EST-7 和 EST-10 只在部分雌性个体中出现。

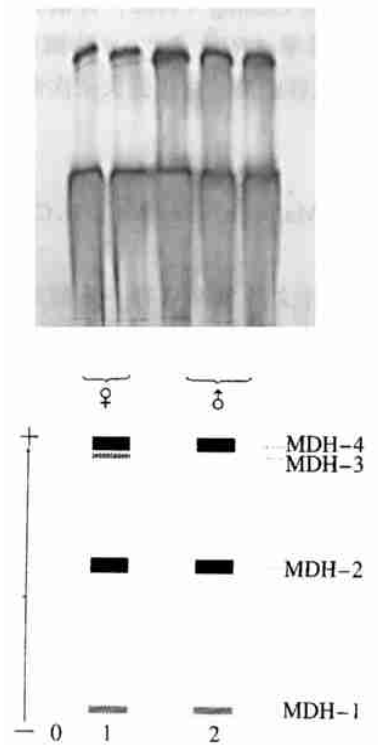


图 2 雌雄文昌鱼苹果酸脱氢酶同工酶的电泳酶谱

0: 原点。1: 雌文昌鱼的苹果酸脱氢酶同工酶的电泳酶谱。2: 雄文昌鱼的苹果酸脱氢酶同工酶的电泳酶谱。MDH-1-MDH-4: 苹果酸脱氢酶表达位点 1 至 4。

Fig. 2 Electrophoretogram of malate dehydrogenase isozymes in male and female Branchiostoma belcheri tsingtauense

0: Origin. 1: The electrophoretogram of malate dehydrogenase isozymes in female. 2: The electrophoretogram of malate dehydrogenase isozymes in male. MDH-1 to MDH-4: Loci 1 to 4 expressed for malate dehydrogenase.

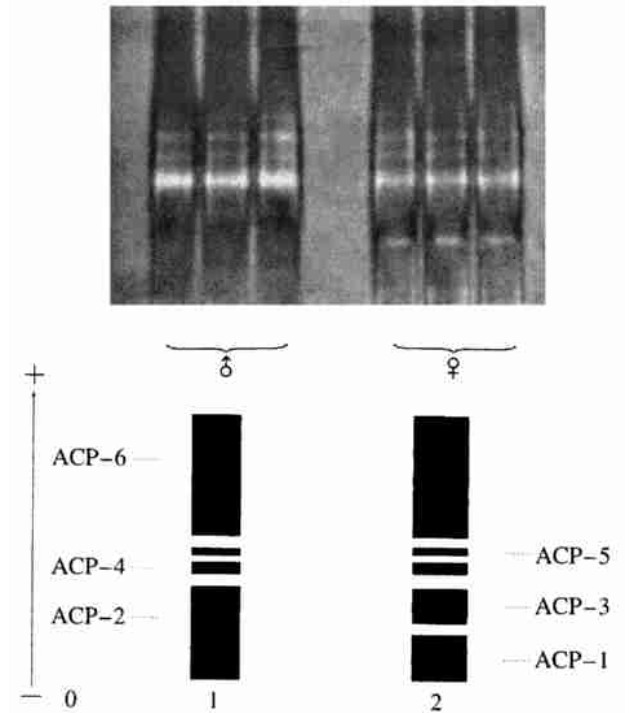


图 3 雌雄文昌鱼酸性磷酸酶同工酶的电泳酶谱

0: 原点。1: 雄文昌鱼的酸性磷酸酶同工酶的电泳酶谱。2: 雌文昌鱼的酸性磷酸酶同工酶的电泳酶谱。ACP-1 to ACP-6: 酸性磷酸酶表达位点 1 至 6。

Fig. 3 Electrophoretogram of acid phosphatase isozymes in male and female Branchiostoma belcheri tsingtauense

0: Origin. 1: The electrophoretogram of acid phosphatase isozymes in male. 2: The electrophoretogram of acid phosphatase isozymes in female. ACP-1 to ACP-6: Loci 1 to 6 expressed for acid phosphatase.

表 1 文昌鱼苹果酸酶、苹果酸脱氢酶和酸性磷酸酶表达位点

Table 1 Loci expressed for the isozymes, malic enzyme, malate dehydrogenase and acid phosphatase in amphioxus

性 别 sex	研究个体 Number of individuals	苹果酸酶 Malic enzyme, ME		苹果酸脱氢酶 Malate dehydrogenase, MDH				酸性磷酸酶 Acid phosphatase, ACP					
		ME-1	ME-2	MDH-1	MDH-2	MDH-3	MDH-4	ACP-1	ACP-2	ACP-3	ACP-4	ACP-5	ACP-6
		1	-	E	I	E	-	E	-	E	-	E	E
2	-	E	E	I	E	-	E	-	E	-	E	E	E
3	-	E	E	I	E	-	E	-	E	-	E	E	E
4	-	E	E	I	E	-	E	-	E	-	E	E	E
5	-	E	E	I	E	-	E	-	E	-	E	E	E
6	-	E	E	I	E	-	E	-	E	-	E	E	E
7	-	E	E	I	E	-	E	-	E	-	E	E	E
8	-	E	E	I	E	-	E	-	E	-	E	E	E
1	E	E	I	E	I	E	E	-	E	E	E	E	
2	E	E	I	E	I	E	E	-	E	E	E	E	
3	E	E	I	E	I	E	E	-	E	E	E	E	
4	E	E	I	E	I	E	E	-	E	E	E	E	
5	E	E	I	E	I	E	E	-	E	E	E	E	
6	E	E	I	E	I	E	E	-	E	E	E	E	
7	E	E	I	E	I	E	E	-	E	E	E	E	
8	E	E	I	E	I	E	E	-	E	E	E	E	

E: 表达较强。I: 表达较弱。- : 不表达。

E: Elevated expression. I: Intermediate expression. - : No expression.

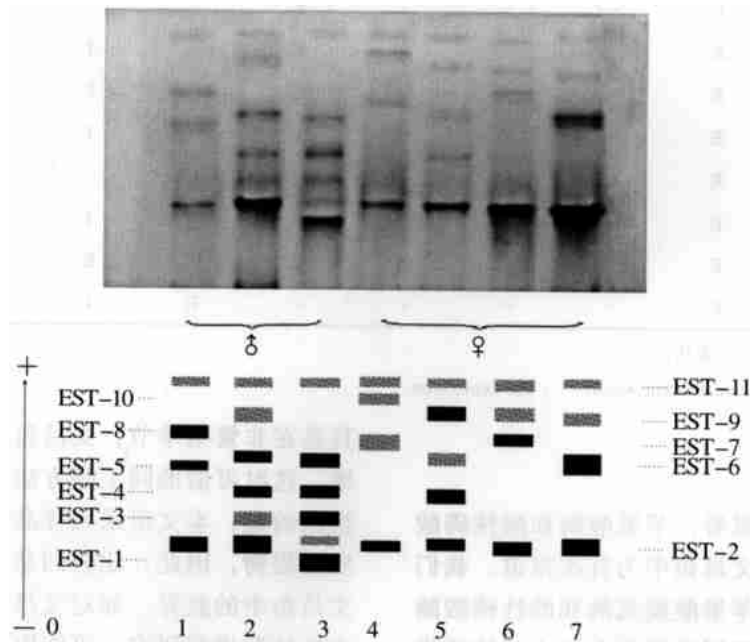


图 4 雌雄文昌鱼酯酶同工酶的电泳酶谱

Fig. 4 Electrophoretogram of esterase isozymes in male and female Branchiostoma belcheri tsingtauense

0: 原点。1 - 3: 雄文昌鱼的酯酶同工酶的电泳酶谱。4 - 7: 雌文昌鱼的酸性磷酸酶同工酶的电泳酶谱。EST-1 - 11: 酯酶表达位点 1 至 11。

0: Origin. 1 to 3: The electrophoretogram of esterase isozymes in male. 4 to 7: The electrophoretogram of esterase isozymes in female. EST-1 to 11: Loci 1 to 11 expressed for esterase.

表 2 文昌鱼酯酶的表达位点

Table 2 Loci expressed for the esterase in amphioxus

性 别 Sex	研究个体 Number of individuals	酯酶表达位点 Loci expressed for esterase										
		EST-1	EST-2	EST-3	EST-4	EST-5	EST-6	EST-7	EST-8	EST-9	EST-10	EST-11
	1	-	E	-	-	E	-	-	E	-	-	I
	2	-	E	I	E	-	E	-	-	I	-	I
	3	E	E	E	E	-	E	-	-	-	-	I
	4	I	I	E	-	-	-	-	E	E	-	I
	5	-	E	-	-	-	I	-	I	-	-	I
	6	E	E	-	-	E	I	-	-	I	-	I
	7	-	E	I	-	-	I	-	-	-	-	I
	8	-	E	-	-	-	E	-	-	E	-	I
	9	-	E	-	-	-	E	-	E	-	-	E
	10	E	E	-	-	-	I	-	-	-	-	I
	11	-	E	-	-	-	I	-	-	I	-	I
	12	I	E	I	E	I	-	-	-	-	-	I
	1	-	E	-	-	-	-	-	I	-	I	I
	2	-	E	-	E	-	I	-	-	E	-	I
	3	-	E	-	-	-	-	-	E	I	-	I
	4	-	E	-	-	-	E	E	-	I	-	I
	5	-	E	-	-	-	E	-	-	E	I	I
	6	-	E	-	-	-	E	-	I	E	-	E
	7	-	E	-	-	-	E	-	E	-	I	E
	8	-	E	-	E	-	I	-	I	-	I	E
	9	-	E	-	E	-	-	E	-	-	-	I
	10	-	E	-	-	-	E	-	I	I	E	E
	11	-	E	-	-	-	E	-	E	E	-	I
	12	-	E	-	-	-	-	E	I	I	-	I

E: 表达较强。I: 表达较弱。- : 不表达。

E: Elevated expression. I: Intermediate expression. - : No expression.

3 讨论

从我们所掌握的文献看, 苹果酸酶和酸性磷酸酶的同工酶电泳谱在文昌鱼中为首次报道。我们还首次发现苹果酸酶、苹果酸脱氢酶和酸性磷酸酶这三种同工酶在文昌鱼不同性别的个体之间酶谱带的数量和迁移率也都存在明显差异, 而在同一性别的不同个体之间则不存在差异。相比之下, 酯酶在文昌鱼中表达情况比较复杂, 它不仅在不同性别之间, 而且在同一性别的不同个体之间都存在差异。因此, 苹果酸酶、苹果酸脱氢酶和酸性磷酸酶电泳谱可以作为一项指标用于文昌鱼的性别鉴定。尤

其是在非繁殖季节, 文昌鱼从外部形态无法分辨雌雄, 这时可借助同工酶方法区分文昌鱼性别。需要指出的是, 本文所采用样品来自整体文昌鱼的蛋白质抽提物, 因此, 比较的是同工酶在不同性别整体文昌鱼中的差异。如对文昌鱼不同组织中同工酶的表达情况进行研究, 可能取得更详细结果。

同工酶都是基因表达的产物, 其表达调控受多种因素影响。已有报道证明, 苹果酸酶基因在两栖类是性别连锁基因, 其表达与性别相关 (Graf, 1989; Wright and Richards, 1993), 而苹果酸脱氢酶、酸性磷酸酶和酯酶的表达可能受不同的生理状态或激素调节影响 (Allen and Moore, 1966;

Raftell and Berzins, 1977)。至于苹果酸酶、苹果酸脱氢酶和酸性磷酸酶在文昌鱼雌、雄个体之间表达差异是受性别连锁基因控制或是受激素和不同生理状态控制，尚待深入研究。

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