

单环刺螯虫变态前幼虫发育的同工酶及酶学研究

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摘要:对实验室条件下培养的单环刺螯虫(*Urechis uniconctus*)变态前幼虫发育的同工酶及酶学特性进行研究。研究中5种同工酶(EST, MDH, AMY, LDH, ALP)酶谱均随发育表现出一定的差异。EST酶谱最为复杂,由原肠期的1条酶带增至体节幼虫时期的6条。MDH在发育各时期皆有表达。在原肠时期未检测到AMY、LDH及ALP活性。采用生化法对5种酶(ACP, ALP, PO, POD, CarE)的酶活测定结果显示,随着单环刺螯虫的发育,各种酶的活力都有明显增强。以上结果表明,摄食行为的开始对其某些消化酶活性的表达有显著的增强作用。至中期担轮幼虫时期,幼虫已具备了一定的对脂类等物质的消化能力。至晚期担轮幼虫及体节幼虫时期,其免疫能力也有显著增强。

关键词:单环刺螯虫;幼虫;同工酶;酶

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在动物早期发育过程中,同工酶谱的变化与形态分化和器官形成具有相关性^[1]。动物的酶学活性是动物摄食、营养条件、生理状况的良好指标^[2]。有关同工酶和酶学的发育研究已在许多动物中作了描述^[1-5],但有关单环刺螯虫(*Urechis uniconctus*)发育的同工酶和酶学研究,尚未见报道。

单环刺螯虫俗称海肠子,属于螯虫动物门(Echiuroidea),螯纲(Echiurida),无管螯目(Xenopneusta),刺螯科(Urechidae),刺螯属(*Urechis*),是一种生活在沿海泥沙岸潮间带下区及潮下带浅水区U型隧道内的底栖生物。本实验利用同工酶及生物化学方法分别对单环刺螯虫早期发育过程中的同工酶和酶学进行研究,以期对单环刺螯虫幼虫发育的营养生理学、水产养殖等提供有益的理论依据。

1 材料与方法

1.1 材料

实验用单环刺螯虫先后于2003年和2004年4月下旬购自青岛四方路海产品市场,体长15~25 cm,外表健康无伤痕。

1.2 方法

1.2.1 实验材料的培养及获取 解剖成熟的雄雌个体,从肾管中获取成熟生殖细胞进行人工授精。

受精卵经洗卵后转入50 cm×30 cm×30 cm的培养缸中培养。培养密度(35±15) ind/mL,水温19~23℃,pH 7.88±0.02,盐度29±1。受精24 h后原肠胚孵化为担轮幼虫,同时开始通气,每隔1~2天换水1次。48 h后开始投喂单细胞藻(小球藻 *Chlorella pyrenoidosa*,角毛藻 *Chaetoceros muelleri*,青岛大扁藻 *Platymonas subcordiformis*,金藻 *Isochrysis galbana*),每天早晚各投喂1次。培养期间,定期观察幼虫的发育状况。本次实验取原肠胚、早期担轮幼虫(消化道刚打通,尚未摄食)、中期担轮幼虫(摄食后生长15~25 d)、晚期担轮幼虫(幼虫下半球伸长,体节幼虫前10 d以后)和体节幼虫(体节出现至附着生活以前)共5个发育时期的样品,分别于-30℃下贮存备用(早期担轮幼虫以后需经48 h的空胃)。

1.2.2 酶学测定 取冰冻的各期幼虫0.1 g,按下述方法(表1)进行生化指标测量,每组实验重复3次。

实验结果用SPSS11.5软件进行one-way ANOVA统计分析。在P=0.05水平上进行差异显著性检验,P<0.05者认为有显著差异,反之则无。

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表1 实验所测量酶的种类及测定方法

Tab.1 Enzymes and biochemical methods

测量成分 Enzymes	方法 Methods
碱性磷酸酶(ALP)	磷酸苯二钠比色法 ^[34]
酸性磷酸酶(ACP)	磷酸苯二钠比色法 ^[34]
过氧化物酶(POD)	Worthington法 ^[37]
酚氧化酶(PO)	Dopa比色法 ^[37]
蔗糖酯酶(CarE)	Hirase法 ^[37]

1.2.3 同工酶 取冰冻的各期幼虫0.1 g,加入甘油-磷酸缓冲液(pH 6.8)3~4滴,冰浴下匀浆,匀浆液于4℃下离心10 min(10 000 r/min),取上清液直接电泳。

采用不连续聚丙烯酰胺凝胶垂直平板电泳法进行同工酶分析,分离胶质量分数为7%~12%,凝胶缓冲液为Tris-cit pH 8.9;浓缩胶质量分数为3%,凝胶缓冲液为Tris-cit pH 6.8;电极缓冲液为pH 8.3的Tris-Gly,采用DYY-IIIA型恒温稳压电泳仪进行电泳,电压200 V,4℃下电泳8~10 h。

显色方法参照文献[3],个别略加改动。显色后的胶片固定于7%的冰醋酸中。Nikon-E4500数码相机拍照。

酶谱示意图参考胡能书等^[4]的方法绘制。酶带的编号参照国际生物化学命名规则而定,以向阳极泳动最快的酶谱带编号为1,如EST-1,并从阳极到阴极依次编号。

2 结果与分析

2.1 变态前5种同工酶随发育的变化

2.1.1 酯酶(Esterase, EST) 在单环刺螈虫早期发育过程中,EST酶谱最为复杂,共6条酶带(图1-①)。其中原肠期只有1条,为EST-4(二级窄带);孵化后(早期担轮幼虫),增至4条,分别为EST-2(二级窄带)、EST-3(三级窄带)、EST-5(二级窄带)、EST-5(一级窄带);摄食后(中期担轮幼虫),除上述4条酶带外,又新产生了EST-1(二级窄带)。且EST-2(一级窄带)、EST-3(二级窄带)活性有所增强,EST-5变为一宽带;至晚期担轮幼虫和体节幼虫,在上述5条酶带的基础上,又新产生了1条酶带EST-6(三级窄带)。

2.1.2 淀粉酶(Amylase, AMY) 单环刺螈虫在变

态前各早期发育过程中,AMY共显示出5条酶带(α -1、 α -2、 β -1、 β -2、 α -3)(图1-②),未检测出R及Q型淀粉酶。除在原肠期未检测到有AMY活性外,在其余4期中,酶带由早期担轮幼虫时期的1条 α -2(二级窄带)增至晚期担轮幼虫和体节幼虫时期的5条: α -1(三级窄带)、 α -2(一级宽带)、 β -1(三级窄带)、 β -2(二级窄带)、 α -3(一级窄带)。

2.1.3 苹果酸脱氢酶(Malate dehydrogenase, MDH) MDH为二聚体酶,分为线粒体型(m-MDH)和细胞质型(s-MDH)。在单环刺螈虫早期发育过程中,原肠期和早期担轮幼虫时期只有1条,为MDH-4(一级宽带)。摄食后谱带数增多,至体节幼虫,共有4条谱带:MDH-1(三级窄带)、MDH-2(三级窄带)、MDH-3(二级窄带)和MDH-4(一级窄带)。其中,MDH-3、MDH-4为m-MDH,活性较强,MDH-1、MDH-2为s-MDH,活性较弱(图1-③)。

2.1.4 乳酸脱氢酶(Lactate dehydrogenase, LDH) LDH在原肠期、早期急中期担轮幼虫时期未有表达,而在晚期担轮幼虫及体节幼虫时期共显示出4条酶带,LDH-1(二级线性带)、LDH-2(二级窄带)、LDH-3(二级宽带)、LDH-4(二级线性带)(图1-④)。

2.1.5 碱性磷酸酶(Alkaline phosphatase, ALP) ALP在单环刺螈虫早期发育过程中表达较为简单(图1-⑤)。在原肠期及早期担轮幼虫时期未检测到ALP活性,在其余3期中ALP-3活性较强,ALP-1与ALP-2为扩散带。

2.2 单环刺螈虫变态前5种酶随发育的酶学变化

2.2.1 碱性磷酸酶(ALP)(图2) 单环刺螈虫孵化前已经具有一定的ALP活性。随发育进行,酶活性持续增强。早期担轮幼虫时期,ALP活性虽有一定增强,但未见明显差异。摄食后一段时间(中期担轮幼虫),活性明显增强。至晚期担轮幼虫及体节幼虫时期,已达到较高水平。

2.2.2 酸性磷酸酶(Acid phosphatase, ACP)(图3)

晚期担轮幼虫之前(原肠、早期担轮幼虫及中期担轮幼虫时期),ACP活性增长较为平缓。晚期担轮幼虫及体节幼虫则有了明显的增强。

2.2.3 酚氧化酶(Phenol oxidase, PO)(图4) 摄食前(原肠及早期担轮幼虫时期),PO活性较低。中期担轮幼虫时期有了一定的增强,至晚期担轮幼虫及体节幼虫时期有了明显升高。

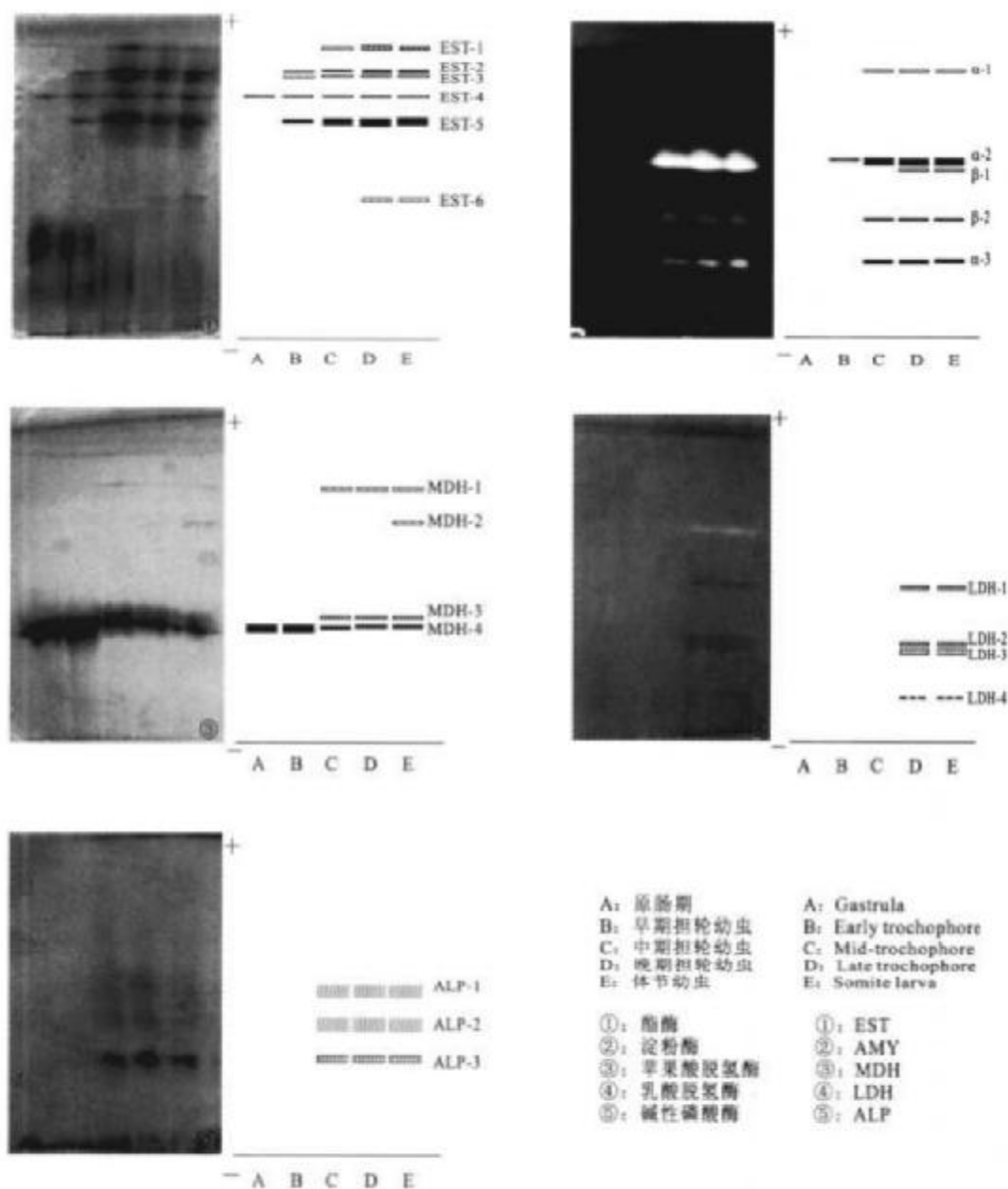


图1 单环刺螵虫不同发育时期5种同工酶图谱

■ 示酶带色深,为一级带; ▨ 示酶带色浅,为二级带; ▩ 示酶带色极浅,为三级带; ▨ 示扩散带。同时每一级酶带又可根据酶带的宽度分为宽带、窄带及线性带(“-----”表示)3类。

Fig.1 Electropherograms of isozymes during development of *Urechis unicinctus*

■ Shows deep isozyme band(the first-level band); ▨ Shows light isozyme band(the second-level band); ▩ Shows extremely light isozyme band(the third-level band); ▨ Indicates the diffused enzyme band. According to the breadth, the enzyme band can be divided into three types; the wide band, the narrow band and the linear band(showed by “-----”).

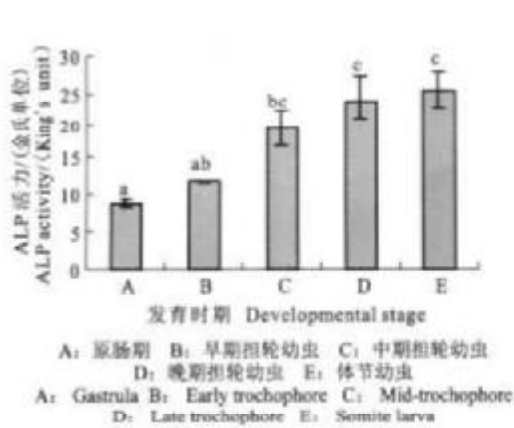


图2 碱性磷酸酶活性随发育时期的变化

Fig.2 Variation of AMP activity with development

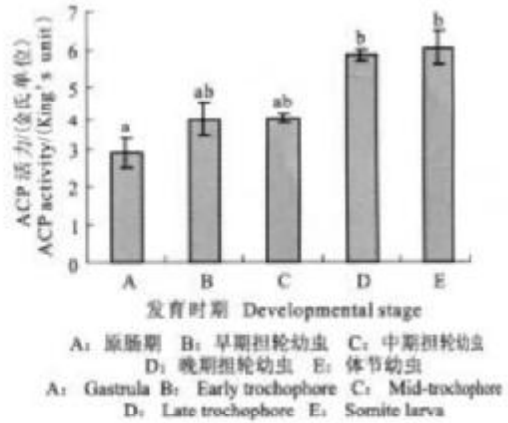


图3 酸性磷酸酶活性随发育时期的变化

Fig.3 Variation of ACP activity with development

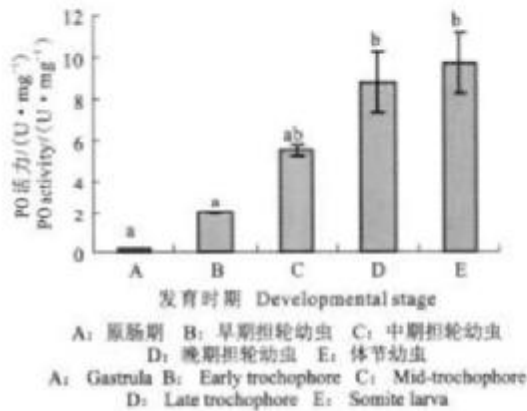


图4 酚氧化酶活性随发育时期的变化

Fig.4 Variation of PO activity with development

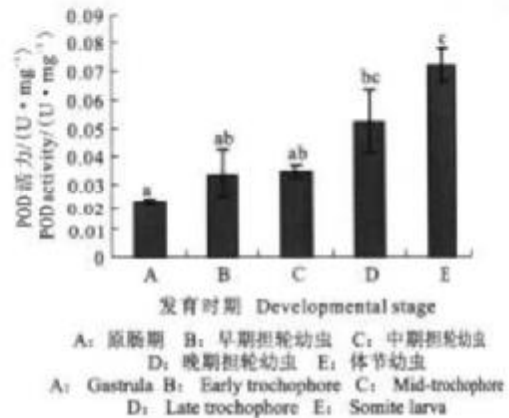


图5 过氧化物酶活性随发育时期的变化

Fig.5 Variation of POD activity with development

2.2.4 过氧化物酶 (Peroxidase, POD) (图5) 晚期担轮幼虫以前,POD 活性增长较为平缓。晚期担轮幼虫及体节幼虫时期,其活性显著增高。

2.2.5 羧酸酯酶 (Carboxylesterase, CarE) (图6) 摄食前(原肠及早期担轮幼虫时期),CarE 活性较低。中期担轮幼虫时期有了一定的增长,晚期担轮幼虫及体节幼虫则增长至较高的水平。

3 讨论

在单环刺螈虫的早期发育过程中,EST、AMY、LDH 等同工酶谱逐渐复杂,这种变化在一定程度上反应了发育过程中细胞的分化。

从本实验的结果来看,单环刺螈虫在早期发育过程中酯酶和淀粉酶的同工酶谱均有一定变化,这

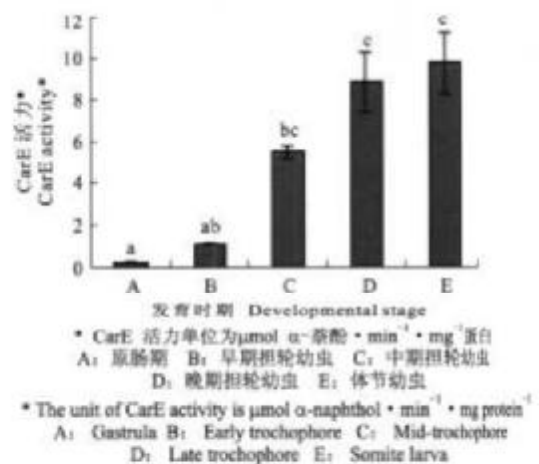


图6 羧酸酯酶活性随发育时期的变化

Fig.6 Variation of CarE activity with development

说明其体内的脂类和碳水化合物代谢随发育有所改变,同时也反映出个体发育对这两类物质需求的不同。根据蒋晓华等^[9]对滇池高背鲫鱼早期胚胎发育期间MDH同工酶的研究,一些MDH同工酶在早期发育阶段是持续表达的,而也有些MDH同工酶仅在某一发育阶段存在。持续表达的酶,可能执行的是最基本的代谢功能。吴力钊等^[10]对同工酶的类型进行了划分,认为在成体组织中较广泛分布,在未受精卵及整个早期发育阶段持续存在的同工酶类,在生物体中担负重要的生理功能,属于管家酶范畴。本实验中的MDH-3属于此范畴,且活性表达较强。碱性磷酸酶是含锌的异型二聚体糖蛋白,活性中心含有丝氨酸。其广泛分布于生物体内,参与机体的脂类和离子等转运活动。本实验中,ALP同工酶谱带较为简单,且只在摄食后的3个时期有所表达,这说明摄食对单环刺螈幼虫ALP同工酶的表达有关键的激活作用。LDH是参与糖代谢的重要酶,属多基因位点同工酶,且此酶与生物环境的含氧量有一定关联。在本实验中,LDH同工酶只在晚期担轮幼虫及体节幼虫时期有所表达。这说明,单环刺螈幼虫到晚期担轮幼虫时期在呼吸及糖代谢功能上有了显著增强。

与目前已报道的无脊椎动物(主要集中于虾、蟹等物种)早期发育过程中同工酶的研究结果比较^[1-2,4-5],单环刺螈在ALP、MDH等同工酶的表达上数量较少,这也在一定程度上显现了此种生物在进化及分类地位上的原始性。

本实验对单环刺螈酶活的生化测定表明,随幼虫发育,其各酶活性都有一定增强。摄食前,5种酶活虽有一定升高,但未见显著差异,此时幼虫生活完全依赖卵黄物质。摄食后,至中期担轮幼虫时期,碱性磷酸酶及羧酸酯酶活性有明显提高。已有报道,碱性磷酸酶活性和脂类、葡萄糖、钙以及无机磷的吸收存在正相关关系^[11],而羧酸酯酶活性的强弱可以间接反映对脂类物质的消化和吸收状况^[11-12]。说明此时幼虫对脂类等物质的消化吸收水平明显增强。酸性磷酸酶是高等动物体内巨噬细胞溶酶体的标志酶,在体内直接参与磷酸基团的转移和代谢,且具有防御和消化的双重作用^[13]。过氧化物酶与细胞膜的保护及细胞衰老有关,同时可将代谢中产生的H₂O₂水解,起到解毒等免疫作用^[14],酚氧化酶更是在无脊椎动物中起着重要的免疫作用^[15]。后2

种酶活,直至晚期担轮幼虫及体节幼虫时期,才表现出显著的增高,这说明单环刺螈幼虫的免疫能力直至此时才真正得到加强。

综上所述,摄食行为的开始对单环刺螈早期发育过程中某些消化酶活性的表达有显著的增强作用。但其免疫能力直至晚期担轮幼虫及体节幼虫才得到显著加强。至体节幼虫时期,其已在消化及免疫方面,为即将到来的附着变态打下了良好的基础。

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Isozymic and biochemical study on development of pre-metamorphic larva in *Urechis uniconctus*

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Abstract: Usually, *Urechis uniconctus* can be found at the sublittoral zone and low area of tideland. In this work, the isozymic and biochemical characteristics of *U. uniconctus* larvae before metamorphosis were studied using the technique of polyacrylamide gel vertical plate electrophoresis and the method of biochemistry. The adult *U. uniconctus* individuals were collected from seafood market of Qingdao during the reproductive season. After a short period of laboratory temporal breeding, the gametes were obtained from the nephridium by dissection, and the artificial semination was performed. The larvae were reared in 50 cm × 30 cm × 30 cm tanks. There were totally 5 larval stages used in this experiment, including gastrula, early trochophore (the digestive canal was just formed and the larvae did not feed), mid-trochophore (15-25 d after feeding), late trochophore (10 d before somite larva) and somite larva (from the formation of somite to the settlement). In the biochemical experiment, the results were analyzed by one-way ANOVA test using SPSS11.5 ($P=0.05$).

The results showed that, all 5 isozymes became complicated with the larval development. EST was the most complicated, increasing from 1 band at gastrula stage to 6 bands at somite larva stage. AMY showed totally 5 enzyme bands, but only α -AMY and β -AMY were found. MDH consisted of two types of isozyme, m-MDH and s-MDH. In this experiment, 4 MDH enzyme bands were found. Thereinto, MDH-3 and MDH-4 were m-MDH, which showed a strong activity. MDH-1 and MDH-2 belonged to s-MDH, and the stain of these two bands was light. At gastrula, early trochophore and mid-trochophore stages, LDH showed no activity. At late trochophore and somite larva stages, 4 enzyme bands could be tested. ALP isozymes showed a comparatively simple electropherogram, and only 3 bands were found at mid-trochophore, late trochophore and somite larva stages. Furthermore, the biochemical study of ACP, ALP, PO, POD and CarE indicated that, the contents of all these 5 enzymes grew higher during early development. Before hatching, the gastrula presented certain ALP activity, until the mid-trochophore, when the activity evidently increased. At gastrula, early trochophore and mid-trochophore stages, the ACP activity was comparatively low, and at the last 2 stages presented a distinct increase. PO activity increased comparatively gently with larvae development. After the late trochophore, the POD activity showed an evident enhancement. As to the CarE, the enzyme activity increased distinctly after mid-trochophore.

During the early development of *U. uniconctus*, the isozyme electropherograms became more and more complicated. This change can reflect the cell differentiation. As the initiation of feeding, most of the isozyme electropherograms showed a certain increase in the quantity of enzyme band, such as the EST, AMY and ALP. And all these 3 enzymes had a certain function in digestion. Totally, these 5 isozymes revealed comparatively simple electropherograms, and this might correlate to the primitivity of the species. The biochemical results inferred that, at the mid-trochophore, the digestive ability in lipid became higher than before, and the immunocompetence of late trochophore and somite larva was obviously enhanced. All the above results indicate that the feeding behavior plays an important role in stimulating the enzymic activity quantitatively and qualitatively.

Key words: *Urechis uniconctus*; larva; isoenzyme; enzyme

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