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KK-42对蜕皮后期日本沼虾头胸甲超微结构的影响

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摘要：为了深入探究KK-42对表皮结构的影响，本实验以外表皮形成趋于结束而内表皮开始出现的蜕皮后期幼虾为材料，通过扫描电镜观察石蜡切片法，分析了KK-42处理前后头胸甲外表皮，尤其是内表皮结构的变化。将处于蜕皮间期的健康幼虾[体长(3.5±0.1) cm]随机分为2组，分别用1.95×10^{-4} mol/L的KK-42溶液和不含KK-42的溶液处理，取蜕皮后1.5、3.0、6.0及12.0 h幼虾头胸甲，观察其超微结构。另取蜕皮后6.0 h头胸甲，用解剖刀轻轻刮去内面组织，通过扫描电镜观察内表皮的表面结构。结果显示，蜕皮后1.5和3.0 h，头胸甲只包含上表皮和外表皮，KK-42处理组外表皮的板层数量显著增加，外表皮厚度分别比相应对照组提高了72.07%和38.67%；蜕皮后6.0~12.0 h，外表皮中疏松的板层趋于致密，其厚度在二组间无统计学差异。蜕皮后6.0 h，只有1层板层的内表皮出现，到蜕皮后12.0 h，板层数增至2层(对照组)和3层(处理组)，且板层结构均疏松。此外，内表皮表面分布着大小不等、头尾走向的梭形孔道(pore canals)；KK-42处理组内表皮结构未见明显变化。实验表明，KK-42对蜕皮后期日本沼虾幼虾头胸甲的超微结构有显著影响，能加快外表皮及内表皮板层的形成速率。

关键词: 日本沼虾；KK-42；蜕皮后期；头胸甲；扫描电镜

中图分类号: S 917.4　　**文献标志码:** A

甲壳动物的表皮(又称外骨骼)具有支持、保护机体的作用，但缺乏延展性，故动物生长发育的显著特征是周期性的蜕皮，一般分为蜕皮前期、蜕皮期、蜕皮后期和蜕皮间期[1]。对蜕皮周期形成机制的研究历史悠久，报道较多[2-5]，各种分子的作用都是通过直接或间接影响旧表皮的降解或新表皮的形成来实现。目前，甲壳动物表皮结构在蜕皮周期中的变化规律已基本明确[6-8]，其中，占据蜕皮周期大部时间的蜕皮间期和蜕皮前期的表皮结构研究最为深入，这是由于蜕皮间期表皮发育最为完善，由外而内的上表皮、外表皮和内表皮结构完整[9]。而蜕皮前期是表皮新旧更替、结构变化最为剧烈的时期，该时期旧表皮逐渐降解，新表皮中的上表皮和外表皮开始形成[10-12]。蜕皮后期持续时间相对较短，是外表皮的形成及钙化作用趋于完成、内表皮开始出现的重要阶段。

本团队前期研究发现，咪唑类物质KK-42处理可以明显缩短日本沼虾(*Macrobrachium nipponense*)幼虾的蜕皮周期[5]，增加蜕皮前期的晚期(D_3)头胸甲内表皮的厚度[12]，推测，表皮厚度的变化可能影响蜕皮周期的时程。本实验以蜕皮后期幼虾头胸甲表皮为材料，通过扫描电镜观察石蜡切片，分析了KK-42对蜕皮后期外表皮，尤其是内表皮超微结构的影响，旨在为阐明KK-42缩短蜕皮周期的机制提供理论支持。

1　材料与方法

1.1　实验材料

日本沼虾捕捞于河南原阳黄寺渔场，饲养

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于水族箱中，每天早晚各投喂1次，1周后选取60尾处于蜕皮间期、体长(3.5±0.1) cm的健康幼虾，随机分为对照组和处理组。处理组用1.95×10^{-4} mol/L的KK-42溶液浸泡1 min[13]后迅速取出，逐只放于水族箱的网格中，每3.0 h观察一次。对照组以不含KK-42的溶液同样方法处理。

1.2 实验方法

头胸甲超微结构的观察 取蜕皮后1.5、3.0、6.0和12.0 h的幼虾，每个时间点取3只，将头胸甲背部剪成5~6 mm的小块，10%甲醛溶液固定36 h，细水冲洗1 h，梯度酒精溶液脱水(在80%酒精脱水前进行苏木精-伊红(H.E)染色，便于后期包埋)，正丁醇透明过夜，石蜡包埋，切片(厚度8 μm)烘干，60 °C的二甲苯中脱蜡3 d，放于玻璃真空干燥器备用。将干燥后的切片喷金，置于JSM-7800F扫描电镜下观察。

内表皮表面结构的观察 取蜕皮后6.0 h幼虾头胸甲，体视显微镜下用解剖刀轻轻刮去内面组织，0.1 mol/L磷酸缓冲液(PBS)冲洗，2.5%戊二醛固定12.0 h，后续处理参照杜娟等[8]方法，将处理好的材料直接喷金，JSM-7800F扫描电镜观察。

2 结果

2.1 KK-42处理对蜕皮后期头胸甲外表皮超微结构的影响

蜕皮后1.5和3.0 h，头胸甲只有上表皮和外表皮，上表皮是一匀质的蜡状层，其下方与之平行且排列较为疏松的多板层结构为外表皮(图版-1，2)；随着时间的延长，外表皮的板层由疏松变得致密(图版-3，4)，厚度先增加后逐渐降低(图1-a)。KK-42处理能明显加快外表皮的形成速率，蜕皮后1.5~3.0 h，外表皮的板层数量明显增多(图版-5，6)，其厚度分别比相应对照组提高72.07%和38.67%(P<0.01)，蜕皮后6.0~12.0 h无统计学差异(图1-a)。

2.2 KK-42处理对蜕皮后期头胸甲内表皮超微结构的影响

蜕皮后6.0 h，对照组和处理组头胸甲只有1层内表皮生成(图版-3, 7)；到12.0 h内表皮板层数量分别增加至2层(图版-4)和3层(图版-8)，且板层均呈疏松状结构，两组间内表皮厚度无显著性差异(图1-b)。刮去头胸甲内面组织以暴露出内表皮，扫描电镜下观察到内表皮表面分布有大量孔道(pore canals)，其形态多呈大小不等、头尾走向的梭形(图2)，KK-42处理组内表皮结构未发现明显的变化。

3 讨论

利用扫描电镜研究甲壳动物表皮结构，一般采用冰冻断裂技术制备材料，但该技术适用于体型较大的动物如中华绒螯蟹(*Eriocheir sinensis*)[14]等。日本沼虾幼虾个体小、表皮薄，

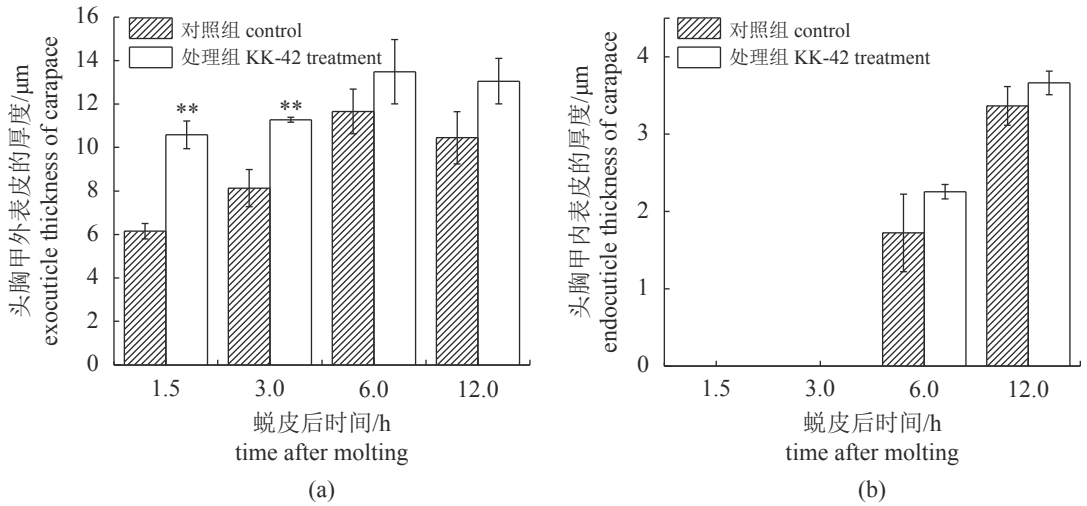

图 1 KK-42对日本沼虾蜕皮后期头胸甲外、内表皮厚度的影响

(a) 外表皮，(b) 内表皮；**. 表示与相应的对照组比有极显著差异(P<0.01)

Fig. 1 Effect of KK-42 on thickness of carapace exocuticle or endocuticle in *M. nipponense* during postmolt

(a) exocuticle, (b) endocuticle; **. represents extremely significant difference (P<0.01) *vs.* control group at the same time

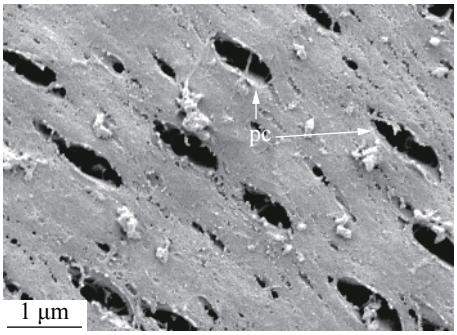

图 2　蜕皮后6.0 h日本沼虾头胸甲内表皮超微结构

pc. 孔道

Fig. 2　The ultrastructure of carapace endocuticle from *M. nipponense* at 6.0 h after molting

pc. pore canals

很难平行于表面断开暴露出外表皮和内表皮，故本实验采用扫描电镜观察石蜡切片法。

研究表明，甲壳动物外表皮的形成与钙化不同步，蜕皮后与钙化相关的表皮蛋白开始表达，钙盐在蜕皮后3.0 h发生沉积，一直持续到蜕皮后5.0~8.0 h[15-16]，这与日本沼虾蜕皮后外表皮板层由疏松变为致密(图版-3, 4)的时间基本一致，推测钙盐的不断沉积使板层结构趋于致密。本实验首次发现，KK-42处理明显加快了外表皮的形成速率，板层数量及厚度显著高于对照组(图1-a)。几丁质与蛋白质是组成外/内表皮的主要物质，本课题组前期研究显示，日本沼虾表皮几丁质合成酶基因在蜕皮前期、后期高表达[17]且KK-42能诱导该基因表达；同时，KK-42能显著上调表皮蛋白基因在蜕皮间期[18]、蜕皮前早期[18-19]的表达，这与新外表皮形成加快的结果相符。

结果发现，在蜕皮后6.0 h内表皮开始生成(图版-3, 7)，构成内表皮的板层较为疏松，这与蜕皮间期内表皮的结构相似[8]，提示，内表皮在形成过程中结构变化不大。KK-42处理同样加快了内表皮板层的形成速率(图1-b)，这应该与KK-42能上调表皮蛋白在蜕皮前期[20]、蜕皮后期[21]的表达量有关。有趣的是，KK-42处理组内表皮厚度与对照组相比并无显著性差异，与前期结果不一致[12]，原因可能是观测时间点不同造成的，本实验选择的是蜕皮后期内表皮刚开始形成，虽板层数增加但太薄，所以内表皮厚度的统计数据无明显差异；而前期研究选择的是蜕皮前晚期，内表皮已有一定的厚度，故二组间差异显著。孔道在物质运输和表皮的矿化作用中起重要作用[7]，本实验对内表皮的表面进行了观察，发现孔道多呈梭形，这与已报道的内表皮孔道形状基本一致[8, 22]，推测，应与几丁质—蛋白质纤维的收缩[23]和表皮中钙盐的不断沉积[8]有关。

综上，KK-42处理能显著影响蜕皮后期日本沼虾幼虾头胸甲的结构，加快外、内表皮的形成速率，这可能是KK-42缩短蜕皮周期的机制之一。

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Effect of KK-42 on the carapace ultrastructure in *Macrobrachium nipponense* during postmolt

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Abstract: In order to further investigate the effect of KK-42 on cuticle structure, the juvenile prawns (*Macrobrachium nipponense*) in postmolt stage during which the formation of exocuticle gradually ends but that of endocuticle starts, were employed to study the structures of carapace exocuticle and endocuticle using the observation of paraffin section by scanning electron microscopy (SEM). Healthy intermolt *M. nipponense* with body length of (3.5±0.1) cm were randomly divided into two groups. The *M. nipponense* were soaked for 1 min in KK-42 solution at a concentration of 1.95×10^{-4} mol/L (treatment group) or 0 mol /L (control group), respectively. The carapaces of *M. nipponense* at 1.5, 3.0, 6.0 and 12.0 h after molting were obtained to be used for ultrastructural observation. To observe directly the surface structure of endocuticle, the carapace obtained at 6.0 h after molting was scraped gently with an anatomical knife to remove tissues on the inner surface of carapace, and then examined using SEM. The results showed that the carapace was only composed of the epicuticle and exocuticle at 1.5 and 3.0 h after molting. The number of exocuticle lamellae derived from KK-42 treatment group rose significantly at 1.5 and 3.0 h after molting, and the thickness of exocuticle increased by 72.07% and 38.67%, respectively, compared with the corresponding control group. At 6 and 12 h after molting, the loose lamellae in exocuticle tended to be dense, and there was no significant difference in thickness to be found between two groups. The number of endocuticle lamellae with a loose structure, being only one at 6.0 h, separately increased to two in control group and three in treatment group at 12.0 h after molting. In addition, pore canals within the endocuticle presented different sizes and head-tail orientation. No significant structural change was observed in the endocuticle after KK-42 treatment. The results reveal that KK-42 has a significant effect on the carapace ultrastructure of juvenile *M. nipponense* during postmolt, and accelerate the formation rate of the exocuticle as well as endocuticle.

Key words: *Macrobrachium nipponense*; KK-42; postmolt; carapace; scanning electron microscopy (SEM)

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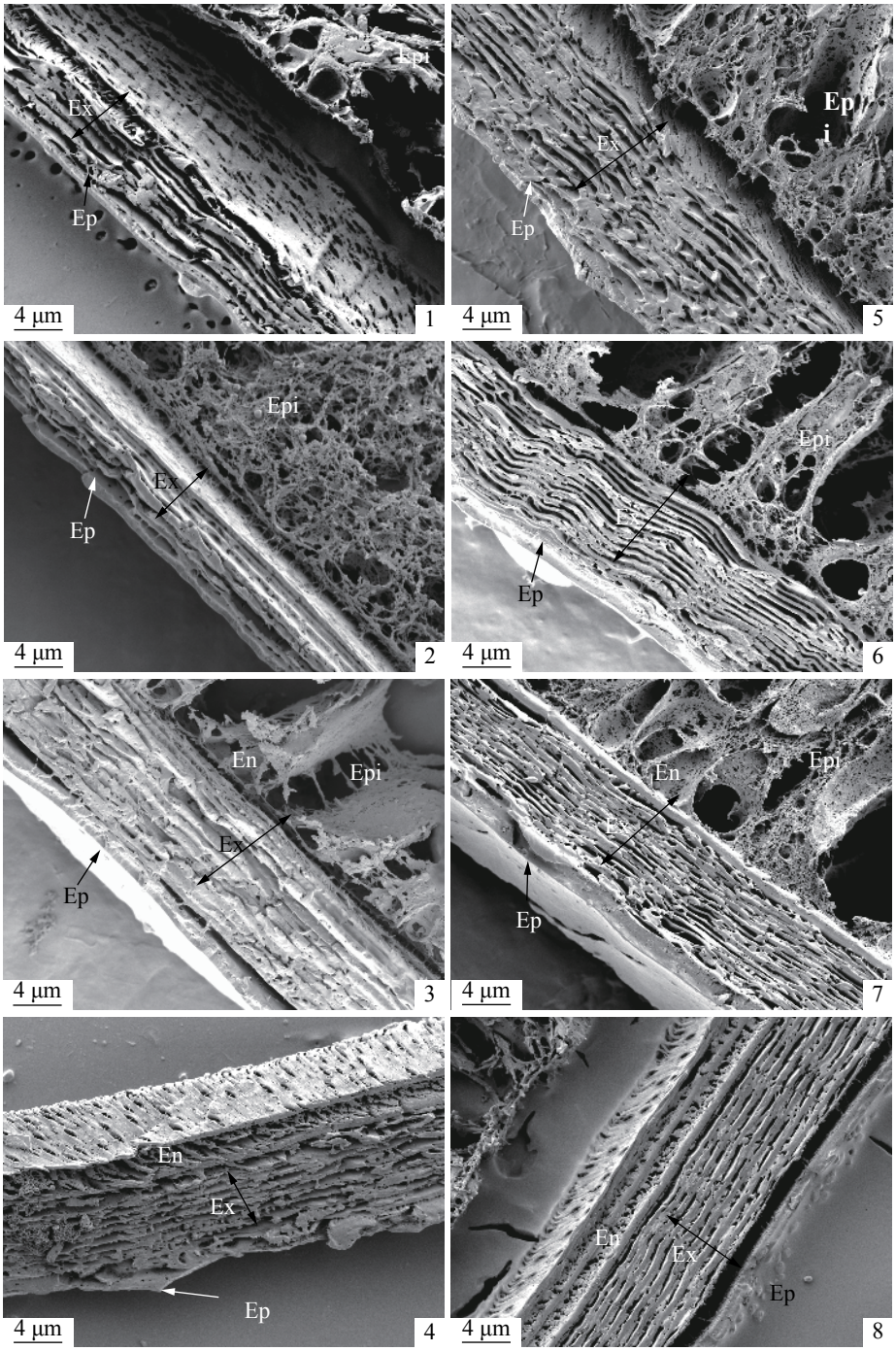

图版 KK-42对日本沼虾蜕皮后期头胸甲超微结构的影响

1~4. 对照组，分别为蜕皮后1.5、3.0、6.0、12.0 h；5~8. 处理组，分别为蜕皮后1.5、3.0、6.0、12.0 h；Epi. 上皮细胞；Ep. 上表皮；Ex. 外表皮；En. 内表皮；pc. 孔道

Plate Effect of KK-42 on the carapace ultrastructure in *M. nipponense* during postmolt

1-4. control group, showing 1.5, 3.0, 6.0, 12.0 h after molting, respectively; 5-8. treatment group showing 1.5, 3.0, 6.0, 12.0 h after molting, respectively; Epi. epidermis; Ep. epicuticle; Ex. exocuticle; En. endocuticle; pc. pore canal