An AMP-ligase with protoluciferase activity: cloning, sequence analysis and properties

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The origin of luciferases, the enzymes that catalyzes the bioluminescence reactions, is one of the most intriguing questions about bioluminescence. Among Coleoptera, recent studies showed that their luciferases originated from Acyl-CoA ligases. However, the evolutionary link between non-luminescent AMP-ligases and luciferases is still unknown. Several years ago, a luciferase-like enzyme, able to produce weak chemiluminescence in the presence of firefly D-luciferin and Mg/ATP, was discovered in *Tenebrio* mealworms, but the detailed tissular origin and its identity remained unknown. Using sensitive CCD Luminescence Imaging of *Zophobas morio* mealworm (Coleoptera: Tenebrionidae), we have found that this enzyme is located in the Malpighian tubules. Therefore we constructed a cDNA library from Malpighian tubules and isolated a weakly luminescent AMP-ligase. The recombinant enzyme displays weak luminescence in presence of D-luciferin and MgATP, with a peak at ~600nm. It is composed to 529 amino acids residues with N-terminal and C-terminal endoplasmic reticulum membrane signal sequences. This luciferase-like enzyme shows high identity with some *Tribolium castaneum* (51-65%) and *Tenebrio molitor* (29-51%) ligases; and unexpectedly, only 26-32% of identity to beetle luciferases. The K_M values for firefly D-luciferin and ATP are 260 µM and 860 µM, respectively, indicating lower affinity for these substrates. The location of this enzyme in the Malpighian tubules, which are involved excretion and metabolization of carboxylic substrates, and the similarity with Benzoate-CoA ligases, suggest that this enzyme could be involved in the excretion of some carboxylic compounds or detoxification of xenobiotics.
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