Suitability of Two Brazilian Beetle Luciferases for Bioanalytical ATP Assays

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Firefly luciferases catalyze the ATP activated oxidation of firefly luciferin, producing oxyluciferin and bioluminescence with high efficiency. Firefly luciferases are actually very important bioanalytical reagents for ATP measurements. However, commercial reagents and kits currently use North-American *Photinus pyralis* firefly luciferase and few other firefly luciferases from Europe and Japan: there are not national kits or reagents in the our market. Therefore we analyzed the suitability of different beetle luciferases (*Phrixothrix spp, Pyrearinus termitilluminans, Macrolampis sp2*), previously cloned in our laboratories for their potential use as bionalytical reagents. The luciferases of *Macrolampis* sp2 firefly and *Pyrearinus termitilluminans* larval click beetle, were the most stable. *Macrolampis* firefly luciferase displays fast flash-like kinetics and the lowest K_M for ATP (60 µM) when compared with previously cloned beetle luciferases from *P. termitilluminans* (250 µM), *Photinus pyralis* (Lampyridae) (250 µM), *Phrixotrix viviani* (330 µM) and *P. hirtus* (Phengodidae) (130 µM). These properties, associated with its high thermal stability, make this firefly luciferase especially suitable for bioanalytical purposes. Therefore we selected this enzyme for analytical assays of ATP in different cells. A standard ATP curve was constructed and the ATP content was assayed for bacterial and yeast cells. The sensitivity of the assay using partially purified enzyme was 2 µM of ATP using a low sensitivity photometer (TDIII). By using more sensitive apparatus, purer and more concentrated preparations of luciferase the sensitivity can be increased orders of magnitude. The selection of *Macrolampis* firefly luciferase constitutes an important step for the development of analytical reagents for the national market. Keywords: ATP, bioanalytical, luciferases. Acknowledgements: CNPq and FAPESP.