**In Situ** Microscopic Visualization and Quantification of Inorganic Polyphosphate Stores by 4',6-Diamidino-2-Phenyldione (DAPI)-staining

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Inorganic polyphosphate (polyP) is a widely distributed biological polymer composed of phosphate residues linked by phosphoanhydride bonds. They play key biological roles such as phosphate and energy reservoir, metal homeostasis, regulation of transcription factors and transcription fidelity, as well as regulation of several enzyme activities [1]. Nevertheless, polyP has remained poorly studied mainly due to the limited methods for polyP quantification and in situ visualization. 4',6-diamidino-2-phenylindole (DAPI) is a commonly used nuclei stain that binds to double stranded DNA, exhibiting a fluorescence emission maximum around 450 nm. Interestingly, it has been shown that polyP is able to shift DAPI emission maximum to a higher wavelength around 525-550 nm displaying a greenish-yellowish color distinct from the blue nuclei-signals [2]. This DAPI-polyP interaction has been shown to be specific and to generate proper quantum yield for microscopic observation. Nevertheless, although a few studies have focused on the spectroscopic properties of analytical grade sodium polyphosphate-DAPI fluorescence, there are no consistent reports concerning methods of polyP visualization using DAPI. In addition, the lack of a general protocol for polyP staining hinders its wider utilization as a polyP-visualization tool. In the present study, we report a systematic evaluation of different protocols of DAPI-staining for the detection of polyP stores in different biological models. Suspensions of subcellular fractions of insect eggs and parasites containing polyP granules showed a clean DAPI-polyP fluorescence after brief incubation with DAPI, without previous treatments. Staining of Poly P stores in intact cells usually required aldehyde fixation and/or detergent permeabilization, as exemplified in DAPI-polyP fluorescence obtained from *Eimeria* parasites. DAPI-polyP signals were also detected from cryosections of mild-fixed, OCT-embedded midgut of insects. In addition, a DAPI-polyP fluorimetric method was applied to quantify polyP mobilization during early egg development of the insect *Rhodnius prolixus*. The method was validated by the parallel measurement using a more widely accepted polyP quantification protocol that uses a recombinant yeast exopolyporphatase. Taken together, our results strongly support the broader use of DAPI as a valuable tool for both polyP visualization and quantification in different biological models. Nevertheless, one should take into account that sample preparation may vary according to the model used and that care should be taken when localizing and quantifying polyP from different biological models.
