Development of novel peptide nucleic acid (PNA) probes for the rapid identification of pathogens by fluorescence in situ hybridization (FISH)

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Peptide nucleic acid (PNA) molecules are DNA mimics, where the negatively charged sugar-phosphate backbone is replaced by an achiral, neutral polyamide backbone formed by repetitive units of N – (2-aminoethyl) glycine²³. Due to their superior hybridization properties, PNA probes to detect pathogens by fluorescent in situ hybridization (FISH) have been challenging DNA probes over the last few years (e.g. 1). In this work, we have designed and developed three new probes for the specific detection of Enterobacter sakazakii, Staphylococcus epidermidis and Salmonella spp. Probes were tested against several related species, and were shown to be specific for the microorganisms of interest. All three techniques were optimized in slides and then adapted for different types of samples, depending on the microorganism: E. sakazakii is a major contaminant of milk-based powdered infant formulas detection, and as such a membrane-based method to detect the pathogen after filtration of contaminated milk was devised; Staphylococcus epidermidis, which is frequently present on the skin of humans, had methods developed for its identification in contact lenses and catheters; and locations of interest for Salmonella spp. included pipes of drinking water distribution systems. Future work with PNA probes will involve multiplexing experiments (i.e. simultaneous detection of several species in a single sample) and application of flow cytometry to compare fluorescent intensities.


Functional insulin quantification upon its nanoencapsulation

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Insulin is the most important drug used in the therapeutics of diabetes mellitus, a metabolic disorder with increasing prevalence. Several alternative routes of administration, other than the subcutaneous one, are currently under development, in order to improve patients’ quality of life¹. Among them, insulin encapsulation within biodegradable polymer particles regarding its oral administration constitutes a promising strategy². In the present study, an in vitro methodology was developed so as to evaluate the preservation of the hormone’s functionality following its submission to nanoencapsulation. The methodology was optimized using commercially available insulin, through rat L6 myoblasts stimulation with different hormone concentrations and for distinct periods of time. Detection of Akt phosphorylated form through Western blotting assays was used as an indicator of an effective stimulation by insulin and, thus, of the retention of its active conformation³. Akt/PKB, one of insulin signalling pathway phosphorylation products, is a protein kinase involved in many of the hormone’s biological actions, including glucose transport and modulation of gene expression. Assays using insulin recovered from nanoparticles revealed that the emulsification/internal gelation technique preserves part of insulin’s functionality. The methodology may be of use as a rapid, ethical, specific and conclusive means of bioactivity screening in pharmaceutical formulations containing the hormone.