SAIB Sociedad Argentina de Investigaciones en Bioquímica y Biología Molecular

CONGRESO SAIB 2024

LX Annual Meeting of the Argentine Society for Biochemistry and Molecular Biology Research (SAIB)

Del 5 al 8 de noviembre de 2024
Pabellón Argentina de la UNC,
Ciudad de Córdoba, Argentina.

EDICIÓN 60

60 años
PROMOVIENDO EL AVANCE DE LA CIENCIA ARGENTINA

transmission electron microscopy (TEM), UV-Vis spectrophotometry, fluorescence spectrophotometry, and Fourier transform infrared spectroscopy (FTIR). TEM analysis revealed that the N-CDs derived from Glu and NADES had spherical shapes with average sizes of approximately 9 nm and 7 nm, respectively. The UV-visible absorption spectra of both Glu and NADES N-CDs displayed absorption bands at 360 nm, corresponding to the π–π* transition of C=C bonds. Fluorescence intensity in the blue/green channel was higher for NADES-derived NCDs compared to those derived from Glu, and under UV irradiation, both types of N-CDs emitted green luminescence. FTIR analysis confirmed that the N-CDs were successfully formed, as their spectra differed from those of the individual Glu, NADES, and bPEI precursors. To evaluate the potential of N-CDs as nanocarriers, the minimum N/P ratio required for effective complexation of plasmid DNA was determined via gel retardation assays. Plasmid size was assessed by electrophoresis after digestion with BsaI, and complexes were prepared using 600 ng of plasmid DNA at varying bPEI concentrations. At N/P ratios equal to or lower than 5.5 (Glu-derived N-CDs) and 1.5 (NADES-derived N-CDs), bands resembling the migration pattern of the free plasmid were observed, indicating that different N/P ratios are required for efficient DNA/N-CD complex formation, with NADES-derived N-CDs showing higher efficiency. Finally, the effect of different N/P ratios of surface-modified CDs on the viability of *S. cerevisiae* was analyzed. The results showed that NADES-derived NCDs were less toxic to yeast cells than Glu-derived N-CDs when dissolved in an aqueous solution. In summary, this work presents a straightforward and cost-effective method for synthesizing cationic CDs with potential applications in DNA delivery during cell transformation.

BT-10

SILK FIBROIN AND CHITOSAN FILMS AS PHOTOSENSITIZER CARRIERS FOR PHOTODYNAMIC THERAPY

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Photodynamic therapy (PDT) is a relatively noninvasive therapeutic method for treating infections and tumors. PDT involves the synergistic use of light, a photosensitizer (PS), and molecular oxygen to generate reactive oxygen species (ROS) and subsequently damage cells. Phthalocyanines (Pcs) have been widely studied PSs. The main disadvantage of Pcs is their lack of water solubility generating aggregates, thus causing photodynamic activity to be lost. To overcome this problem, Pcs can be incorporated into drug delivery systems such as liposomes, nanoparticles, or polymers. In this sense, silk fibroin (SF) and chitosan (Ch) are biocompatible and biodegradable polymers that have attracted attention for their applications in drug delivery. Polymeric films are produced by solvent evaporation and constitute an attractive drug delivery system. The blending of SF and Ch represents a promising approach to developing biomaterials that capitalize on the strengths of both polymers while mitigating their weaknesses. Specifically, the Ch/SF blend exhibits improved mechanical properties, enhanced biocompatibility, and a balanced degradation rate, making it a suitable candidate for several applications. These networks can encapsulate hydrophobic molecules within, protecting them from degradation in physiological environments thereby improving drug solubility in aqueous media. This work evaluated three combinations of Ch/SF (25/75, 50/50, and 75/25) polymeric films as carriers for Zn(II) phthalocyanine (ZnPc) to be used in photodynamic therapy. The photophysical properties, such as UV-vis absorption and fluorescence of ZnPc incorporated into Ch/SF films, were assessed, and the results indicate that all formulations were suitable for loading a hydrophobic PS such as ZnPc. The efficacy of Ch/SF blends as carriers for ZnPc to be used in PDT was evaluated *in vitro* in two cancer cell lines, T98G (glioblastoma) and HeLa (cervical cancer) cells. Three concentrations of ZnPc in each formulation were evaluated: 1, 0.5, and 0.25 ⌈M, with a light dose of 15 J/cm^2. The dark toxicity was evaluated in all cases, and no adverse effects on the cells were observed at the concentrations tested. However, when combined with light, the new formulations showed promise in photoinactivating cells. In summary, these new vehicles demonstrated encouraging features for delivering photosensitizers in PDT.

BT-11

IMMOBILIZATION OF ENVIRONMENTAL BACTERIA USING MICROFLUIDIC DEVICES AND INDUSTRIAL BYPRODUCTS

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Environmental bacteria play a crucial role in regulating ecosystem services and can be utilized in biotechnological products with innovative applications across various fields. Our studies focus on the immobilization of environmental bacteria for use in contaminant removal processes. Lab-on-a-chip technology applied to bacterial immobilization is an emerging area, offering the advantages of system miniaturization and low-cost analysis. The aim of this work was to evaluate the immobilization, through biofilm formation, of bacteria isolated from petroleum-contaminated

environments using microfluidic devices (chips) as a support and a low-cost culture medium formulated with industrial byproducts. The planktonic growth of *Pseudomonas* sp. P26 (P26) and *Rhodococcus* sp. F27 (F27), bacteria selected for their ability to remove petroleum aromatic compounds, was assessed both in LB broth (a standard culture medium) and in the alternative medium based on corn steep water and crude glycerol. Planktonic growth was assessed by enumerating colony-forming units (CFU) per millilitre. In order to immobilize bacteria, polydimethylsiloxane (PDMS) chips with a glass base and an internal chamber volume of 300 µL were fabricated. The biofilm formation of P26 and F27 in the chips (at 30°C, for 72 hours with continuous flow) was evaluated in the low-cost culture medium. Biofilm formation was analyzed using electron microscopy and the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) technique to determine cellular metabolic activity. The growth of planktonic cultures and bacterial immobilization in the chips were successful for both P26 and F27, with P26 forming significantly more abundant biofilms than F27. Both strains developed complex, structured biofilms with intercellular connections and a moderate amount of extracellular material (in P26). These promising results in biomass immobilization on chips, using regional industrial byproducts, pave the way for the design of bioproducts with potential for environmental contaminant removal.

STRUCTURAL BIOLOGY

SB-2

UNRAVELLING THE COMPLEX REGULATION OF MYCOBACTERIAL LARGE GLUTAMATE DEHYDROGENASE: FIRST STEPS IN THE SEARCH FOR mL-GDH$_{180}$ FILAMENTS

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Glutamate dehydrogenases (GDHs) are oligomeric enzymes that catalyze the oxidative deamination of L-glutamate in most living beings. They are classified into two subfamilies: small GDHs (S-GDHs) are hexameric, with monomers of 50 kDa, while large GDHs (L-GDHs) are tetrameric, with subunits of 115 or 180 kDa. S-GDHs have been the subject of diverse biochemical and structural studies while L-GDHs have been less studied.

The first experimental structural model of a bacterial L-GDH$_{180}$ was obtained by our group by X-ray protein crystallography and cryoEM. Based on the modular organization thus revealed for the mycobacterial enzyme (mL-GDH$_{180}$), it is possible to infer an allosteric mechanism of metabolic sensing. Also, we have enough evidence that mL-GDH$_{180}$ forms filaments and we postulate that this attribute has an important functional role. Our goal has been to decipher the molecular basis of the activity and the regulation of mL-GDH$_{180}$ as well as the enzyme ability to form filaments.

We first performed an *in vitro* biochemical characterization of recombinant mL-GDH$_{180}$ and designed and experimental set-up to perform activity measurements in the presence of various metabolites that could possibly modify the oxidation rates by the enzyme. In parallel, by performing a screening of buffers and ionic strengths by size exclusion chromatography we detected oligomeric species of mL-GDH$_{180}$ with stoichiometries higher than the tetrameric form and analyzed how the enzyme activity varied under such conditions. Then we proceeded to perform negative-staining and cryoEM experiments of mL-GDH$_{180}$ to visualize the structure of the enzyme in such conditions.

We will discuss how these results bring us closer to a better understanding of the molecular basis of the complex modulation of mL-GDH$_{180}$activity.

SB-3

CHARACTERIZATION OF A MONOCLONAL ANTIBODY BY MASS SPECTROMETRY

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Biopharmaceuticals are a rapidly expanding class of drugs marked by inherent complexity and variability, necessitating a thorough understanding of the structural attributes that can significantly influence the drug's stability, effectiveness, and safety. A monoclonal antibody (mAb) is a lab-produced molecule engineered to bind to targets with high precision and specificity. In biopharmaceuticals, mAbs are invaluable for treating various diseases, including cancer and autoimmune disorders. Trastuzumab is a mAb used to treat HER2-positive breast cancer by targeting and inhibiting the HER2 protein, promoting cancer cell growth. It is marketed under Herceptin and has become a cornerstone in the global oncology market, significantly improving patient outcomes. Mass spectrometry is essential in mAb characterization, providing detailed analysis of molecular structure, post-translational modifications, and purity, which are crucial for ensuring the efficacy and safety of the final product. In this work, we aimed to characterize trastuzumab preparations using a battery of MS techniques at three levels: (i) intact protein analysis, (ii) middle-down, and (iii) bottom-up proteomics. We used a Q Exactive HF (Orbitrap) mass spectrometer with the BioPharma option (extended mass range