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(In memoriam ***Dr. Juan Carlos Fasciolo****)*

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29.

CAPILLARY ELECTROPHORESIS FOR GLUTATHIONE AND GLUTATHIONE DISULFIDE QUANTIFICATION TO MONITOR OXIDATIVE STRESS BY CADMIUM IN *GLYCINE MAX* LEAVES

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Antioxidant defense systems allow plants to develop a tolerance to contamination with cadmium (Cd). A rapid and accurate capillary electrophoresis (CE) method for the simultaneous determination of GSH and GSSG in *Glycine max* L. leaves was developed. The method was applied to monitor the oxidative stress after Cd adding. Soybean seeds were germinated under controlled conditions. On the 4th day were placed in hydroponic conditions, with Hoagland nutrient solution. On the 10th day were subjected to Cd poisoning (40 µM) for 4, 6, 24, 72 h and 6 days. To obtain extracts, 250 mg of leaves were homogenized under ice-cold conditions in HCl 0.5M. Homogenates were centrifuged for 10 min and the supernatants were analyzed. A Beckman P/ACE MDQ instrument equipped with a DAD and P/ACE System MDQ Software was used. Linear relationships were obtained between corrected peak areas and concentrations of the analytes. Detection was performed at 214 and 198 nm GSH and GSSG were baseline separated in less than 6 min, the corresponding migration times were 4.97 and 5.41 min, respectively. Along the time curve GSH levels were higher than in the respective controls, while GSSG was always lower. GSSG content in the analyzed samples was low and GSH content was high. However, both compounds could be separated and quantified fast and accurately without interference. The increased levels of GSH showed that the antioxidant mechanisms in the plant are working, defending it, and presenting a level of tolerance to Cd stress.

30.

LYSOZYME SEPARATION FROM EGG WHITE BY AFFINITY CROSS-FLOW FILTRATION

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The principle of the affinity filtration relies on the binding of the target protein to a macroligand by specific adsorption and the use of a membrane, which pore size is able to retain only the protein-macroligand complex and allows the passage of other proteins from the mixture. Affinity macroligand for protein purification was prepared from yeast cells modified by chemicals and the Cibacron blue F3GA ligand molecule immobilized to the cell wall by covalent bond. The amount of ligand immobilized to the wall cell was determined by spectrophotometric method. The affinity macroligand and the hen egg white homogenized and diluted were contained in a 2 L stirred bioreactor. A tubular inorganic membrane of 0.45 µm pore size was coupled to bioreactor through a peristaltic pump. The filtrate was collected and the retentate was recirculated to the bioreactor until the achievement of the desired separation. The purity of Lysozyme was assayed by gel electrophoresis (SDS-PAGE). The maximum attachment of ligand on the wall cell was 224 µmol $g^{-1.}$ Preliminary experiments indicated that lysozyme was purified with high purity (more than 80%) from hen egg white. Cibacron Blue F3GA, shows affinity towards NAD^+-dependent enzymes. This is due to the conformational and charge-distribution resemblance between the Cibacron blue and nucleotides.

31.

ADSORPTION OF SERUM ALBUMINS TO IMMOBILIZED CIBACRON BLUE F3GA

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Dye-ligand molecules are know to bind several proteins through the so-called pseudo-biospecific adsorption. The objective of this work is to study the adsorption and selectivity of Cibacron blue F3GA dye with bovine (BSA) and human (HSA) serum albumin. Affinity macroligand from yeast cells modified by chemicals and with the dye-ligand molecule covalently coupled to the cell wall were prepared. The amount of ligand immobilized to the cell wall was determined by spectrophotometric method. Proteins adsorption from serum with Cibacron blue-macroligand was studied in batch-wise at 25ºC. The effect of ionic strength on non-specific adsorption of the macroligand was studied. The purity of BSA and HSA was assayed by gel electrophoresis (SDS-PAGE). The maximum attachment of ligand on the cell wall was 224 µmol g^{-1}. Results of adsorption from serum indicate that BSA and HSA were the main proteins adsorbed. Effect of ionic strength was significant to the BSA and HSA adsorption. Adsorption of other serum proteins on the dye-macroligand was negligible. The affinity macroligand is more effective with human serum than with bovine serum. The adsorption of albumins to the dye is attributed to the structural similarity of bilirubine with the ligand, thus, the ligand occupies the protein site for binding and transport of bilirubine.

32.

PROGESTERONE PRODUCTION OF POLYCYSTIC OVARY IS ASSOCIATED TO CHANGES IN THE MACROPHAGE CYTOKINE EXPRESSIONS IN RAT

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We have showed that progesterone (P) release from rat polycystic ovary (PCO) is modified by secretions of spleen macrophages (MΦ). In this work we study if steroidogenic ability of MΦ secretions from PCO rats is associated to changes in the MΦ cytokine expressions. The PCO was induced in adult rats by a single i.m. injection of estradiol valerate, 2 mg/rat. After 2 months, the rats were sacrificed. MΦ ($1x10^6$ cells) from PCO and no-PCO (control) rats were cultured for 24 h in RPMI medium added with 10% of FBS. Those secretions were used to incubate PCO ovaries for 3 h in metabolic bath. Ovarian P release was measured by RIA and the mRNA levels of 3β-hydroxy esteroide dehydrogenase (3β-HSD) -enzyme of P synthesis- in ovary, by RT-PCR. In MF, the mRNA expression of interleukin (IL) 1β, IL-6, TNFα and IL-10 were determined by RT-PCR, and nitrites (NO) release by Griess reaction. The PCO-MF secretions on PCO ovary increased the P release and 3β-HSD mRNA in ovary, compared with C-MF secretions. PCO-MF showed a higher mRNA levels of IL-6 and TNFα ($p<0.001$) and a lower mRNA expression of IL-1β and anti-inflammatory IL-10 than C ($p<0.01$). NO release from PCO-MF was increased compared with that of C-MΦ. The increase of pro-inflammatory cytokine expressions in PCO-MΦ is involved in the P production from PCO ovary.