H - 61

UTILIZATION OF REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (RP-HPLC) FOR THE ANALYSIS OF RECOMBINANT THYROTROPIN DIRECTLY IN CHO CELL CONDITIONED MEDIUM

de Oliveira J.E., Mendonça F., Peroni C.N., Souza J.M., Bartolini P., Ribela M.T.C.P.

Department of Application of Nuclear Techniques in Biological Sciences, IPEN-CNEN São Paulo, Brazil

CHO-derived recombinant human thyrotropin (rec-hTSH), whose clinical utilization for the diagnosis and therapy of thyroid cancer is well known, is commercially available under the name of Thyrogen^{R} (Genzyme, Cambridge, MA, USA) and has also been synthesized in our laboratory.

For its characterization, a RP-HPLC methodology has been set up, showing it to be a powerful tool for the qualitative and quantitative analysis of this hormone directly in CHO cells conditioned medium.

Utilizing a wide pore C4- bonded silica stationary phase and eluents at neutral pH, an adequate resolution of the protein of interest from CHO contaminant proteins was obtained, even considering the complexity of the crude cell extract.

The results of a validation procedure, carried out utilizing an extract from control host cells, showed that the described methodology is accurate, precise and highly sensitive. The method allows, therefore, a first evaluation of the secreted product before its purification, with the intent of improving the production process by choosing the ideal moment and conditions for the harvesting and purification.

Considering that RP-HPLC separation is based on the hydrophobic properties of the molecules, the methodology here set up can also be used to evaluate this parameter, also important for defining the purification strategy. A first study carried out by comparing pituitary hTSH (obtained from NIDDK, Baltimore MD, USA) and the recombinant products now available (Thyrogen and rec hTSH-IPEN) is showing indeed a significant difference in hydrophobicity, probably due to the different carbohydrate composition of the two molecules.

Supported by FAPESP

H - 62

AN INEXPENSIVE MODIFIED BIOCOMPOSITE ELECTRODE AS MODEL FOR IMMUNOLOGICAL ASSAY APPLIED TO SCHISTOSOMA MANSONI DETECTION

Lima, M. A. [1];Silva, D.C.C. [1]; Coelho, G.D.[1]; Malageño, E.; Lima Filho, J.L.[1,2] and *Dutra, R.F. [1,3]
1 – Laboratório de Imunopatologia Keizo Asami - LIKA / UFPE – Avenida Prof. Moraes Rego, s/n, Cidade Universitária, Recife-PE, CEP: 50670-420; 2 – Depto. de Bioquímica / CCB / UFPE; 3 – Depto. de Patologia / ICB / UPE; * rdutra@hotlink.com.br

The Schistosomiasis is a tropical disease identified using microscopic assay, but this approach is not sensible in chronic stages. The immunosorological assays are more sensible and the ELISA methods are often used. However, these assays require sophisticated equipments and time-consuming steps. This work describes an amperometric model for antibody detection of antibodies *Schistosoma mansoni* in human serum. Comparing to the optical and acoustical techniques, this amperometric biosensor is more specific and allows easier discrimination of non-specific binding. Therefore, a novel epoxy-graphite biocomposite modified electrode using tetracyano-quinodimethane and silver was developed. An antigen solution of *Schistosoma mansoni* (0,5µg/ml) was incorporated into this rigid matrix and cured for 72h at 30°C followed by incubation with 2% casein solution. This working electrode was disposed in the cell against a reference and counter electrodes at 0.26 volts and a FIA system was maintained at 28°C. The results of current response in relation with ELISA absorbance showed significant correlation ($p<0,05$). The optimal dilution of serum was 1:100 similar to ELISA, and it was possible to detect concentration around 1:10000 dilution. Antigen concentration into electrode (0.25 to 10µg/ml) was studied; moreover the optimal peroxide hydrogen concentration to reveal amperometric response was 50mM. This work points the way to the development of a new immunosensor and can be used to diagnostic for others infectious diseases.

Supported by: FACEPE, JICA and CNPq.

H - 63

UREASE AS A SENSITIVE BIOSENSOR FOR THE DETECTION OF INORGANIC AND ORGANIC MERCURY SPECIES IN THE WATER

Araujo, U.C. and Castro Faria, M.V.
Departamento de Biologia Celular e Genética - IBRAG - UERJ, Rio de Janeiro, RJ

Mercury is extremely toxic for mammals and its inorganic form is biotransformed to organic compounds as methyl-mercury which is bioaccumulated in aquatic species (as carnivorous fish). The evaluation of mercury chemical species in the water is important in the detection of contamination sources and in the prevention of health and environmental hazards. Thus, our laboratory is developing a sensitive, simple and low cost enzymatic method based on the ability of urease to be inhibited by very low concentrations of mercury species (Hg^{2+} and Me-Hg). We used a commercial urease preparation (from jackbeans, type III - Sigma cat U-1875). Incubation mixture (3,2 mL) contained enzyme (corresponding to 50µL of 1,33 units) and mercury standards (or deionized water in controls) in presence of 112 mM sodium citrate pH 7,0 and 250 mM phosfate buffer, pH 7,7. After a 30 min preincubation, the enzymatic reaction was started by the addition of 52 mM urea at room temperature and stoped 30 min latter by adding 200µL NaOH 5.0 M. The formed NH_3 was immediately determined by a selective NH_4^+ electrode (Orion Research) coupled to a potentiometer. The eletrode was previously standardized with adequate amonia standards. Inhibition curves showed that 1.0 µg/L Hg^{2+} and 4 µg/L Me-Hg in the water inhibited about 30% the urease activity, showing the high sensibility of the method and the possibility of its application in potable water analysis. 0,1 mM EDTA in the incubation mixture may be used for the differentiation of the inorganic from the organic form, since EDTA is a specific chelating agent for the first species. From twelve metals tested (at 10 ppm level) only Cu^{2+} and Ag^{+} could inhibit urease. Cu^{2+}, however, is completely chelated by the citrate present in the assay. The next step is the elimination of the Ag^{+} interference and to perform paired-analysis with Atomic Absorption Spectrometry using reference standards and real samples.

H - 64

DEVELOPMENT OF SENSOR FOR DETERMINATION OF CHLORIDE IN *Atriplex nummularia*

Carvalho, M.J.S[1].; Oliveira, M.I.P.[2]; Oliveira. I.P.[3]; Lima, F.[1]; Pimentel, M.C.B.[2]; Silva, V. L.[1]

1- Departamento de Eng. Química/Lab. Engenharia Ambiental e da Qualidade-UFPE
2- Departamento Bioquímica/Lab. de Imunopatologia Keizo Asami (LIKA)-UFPE
3- Departamento de Engenharia Civil/Mestrando. Tec. Amb. e Rec. Hídricos-UFPE

mjsc@zipmail.com.br;mariaisaura@zipmail.com.br; barros_carneiro@hotmail.com.br valdinete@hotmail.com; fernandinholima@bol.com.br; ipo@npd.ufpe.br

ABSTRACT

The salinization of soils frequently associated with hydric contamination of the arid and semi-arid zones, has constituted one of the main problems for the development of new species of vegetable (Aupelf, 1999). Soto (2000) affirms that *Atriplex nummularia* is one of the most actuality important species in the reforestation and to the combats against desertification of the arid zone. Besides, these species are easily cultivated, which are formed by green foliage and are well accepted by the cattle (Lailhacar *et al.*, 1989). The chloride determination in this plant leads to a preliminary evaluation of this ion in the arid zone, was made potentiometricaly in FIA system using a tubular electrode according to the procedure describe by Alegret *et al.*, (1984), with a crystalline membrane that is sensitive to chloride (Lima & Rocha, 1990). Standard solutions of NaCl were prepared from appropriate dilutions of a solution 0.1mol/L, in buffer HNO_3 + KNO_3 0.1M and $AgNO_3$ 10^{-5}M, pH 6.0. The electrode showed Nerstian response (slope 51.47±0.19 mV/decade) in the concentration range of 10^{-4} to 10^{-1}mol/L of chloride. The best experimental parameters were: pH 6.0, volume of sample 50µL and flow rate of 3.0mL/min. It was possible to determine 76 samples/h. When this system was used to determine chloride in aqueous plant extract samples which showed $5.3x10^{-4}$mg/Cl⁻/g of sample presented good correlation compared to the reference method (Iodometry) with relative error of 0.99%. The amount of protein in the extract was $3.63x10^{-7}$µg protein/mL.

Supported by: UFPE, CNPq, University of PORTO

H - 65

A RECOMBINANT ELISA TO DIAGNOSE THE BOVINE HERPESVIRUS 1 INFECTION.

A.V. Folgueras-Flatschart. & M.C. Sogayar
Instituto de Química, Universidade de São Paulo - Caixa Postal 26077, CEP 05599-970
São Paulo-SP, Brasil
folguera@iq.usp.br and mcsoga@iq.usp.br

Bovine Herpesvirus type 1 (BHV-1) is associated with different syndromes in bovines, the most common and important ones being genital and respiratory illness. The success of erradication programs of BHV-1 infection in herds depends mostly on correct diagnosis and identification of infected animals. In these programs, indirect diagnostic assays are used to detect antibodies against the virus present in serum. ELISA and Western-Blot assays have been developed with the aim of finding more rapid and unexpensive assays. In this work we tested two recombinant BHV-1 glycoproteins in a recombinant ELISA to detect Bhv-1 antibodies in serum samples.

BHV-1 gC and gD glycoproteins were expressed using the Bac-to-Bac baculovirus expression system (Life Technologies) and Sf9 insect cells. Two types of antigens were prepared from recombinant baculovirus infected insect cells, namely "celular extract" and "nuclear extract", using, respectively, a strong and a mild lysis buffer. Optimal conditions for ELISA were determined by a block titration assay, testing different amounts of protein in solid phase and different dilution factors of a positive and a negative serum, previously determined by a serum-neutralization test. The two types of antigens, but mainly "nuclear extracts", are promising for use in the diagnosis of BHV-1 infection.

Supported by: FAPESP, CNPq, ICGEB, CABBIO, PRP-USP

H - 66

CONSTRUCTION OF INTERNAL STANDARDS FOR EVALUATION OF mRNA LEVELS OF THE INTERFERON ALPHA/BETA RECEPTOR

Carlos E. Melo[1], Mario H. Hirata[1], Nga Y. Nguyen[2] and Rosario D. C. Hirata[1]
[1]Department of Clinical and Toxicological Analysis, Faculty of Pharmaceutical Sciences, University of Sao Paulo, Sao Paulo, SP, Brazil; [2]Center for Biological Evaluation and Control, Food and Drug Administration, Bethesda, MD, USA.

Interferon alpha/beta receptor (IFNAR) mRNA expression patterns in the liver have been shown to correlate with the effectiveness of Interferon alpha therapy of patients with hepatitis C virus (HCV) infection. In this study, we present two strategies for construction of internal standards useful in competitive reverse transcription-polymerase chain reaction (RT-PCR) assays to measure mRNA level of the human IFNAR subunits 1 and 2 (IFNAR1 and IFNAR2). Total RNA was extracted from peripheral mononuclear cells and cDNA was prepared using 1 µg of total RNA. Both IFNAR1 and IFNAR2 cDNAs were amplified by PCR and inserted into *Bam*HI-*Eco*RI cloning sites of pBluescript KS(-), pB, using different strategies. The internal standard for IFNAR1 was constructed by the cloning of a 504-bp amplified fragment of the IFNAR1 cDNA into pB. A deletion of a 43-bp internal sequence of the inserted fragment was carried out by enzymatic restriction with *Nde*I and *Dra*I, producing the recombinant plasmid pB-IFNAR1-1. The internal standard for IFNAR2, pB-IFNAR2-2, was prepared using site-directed mutagenesis by overlap extention PCR, resulting in a 236-pb product that was cloned into pB. The recombinant plasmids were introduced into *E. coli* DH5-α competent cells. Plasmids were extracted from isolated transformands and analyzed by enzymatic restriction with *Bam*HI and *Eco*RI, and by PCR amplification. Fragments corresponding to the modified inserts cloned into pB were confirmed by agarose gel electrophoresis. Using these cloning strategies, we have successfully constructed internal standards that can greatly improve the accuracy of the RT-PCR procedures used for the evaluation of the IFNAR mRNA expression levels.

Financial Support: FAPESP-Brazil