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POSTER PRESENTATIONS

Biochemical Genetics: Methodology

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Simplified approach to screening of hemoglobin variants using anion-exchange high performance liquid chromatography. M. Naveed, S. Agarwal and S.S. Agarwal*, Department of Medical Genetics, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Post Box 375, Lucknow-226001, India.

Of the several structural variants of hemoglobin seen in Indian sub-continent; Hemoglobins E, D-Punjab, and S are more common. They frequently interact with beta-thalassemia. In the reported literature, separation of above variants from normal hemoglobins A, A2 and F requires use of different columns and buffer systems. Mostly cation-exchange columns have been used for this purpose. We have developed a single anion-exchange system to separate normal and abnormal hemoglobins seen in the Indian sub-continent. It consists of a 6x10 mm TSK-DEAE-5PW guard column and a 7.5x75 mm TSK-DEAE-5PW anion-exchange column (Hydrophilic Polymer Gel, 10 um particle size). The chromotogram is developed using a gradient between 0 to 0.3 M NaCl in Tris (0.015 M) - Potassium Cyanide (0.0015 M) buffer, pH 8.58. With a run of 30 minutes, the retention time of A2, A and F were 10.6, 16.7 and 22.3 minutes respectively. For separation of hemoglobins E, D-Punjab and S from normal hemoglobins A and A2, a slower gradient over 120 minutes is needed.

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RECOVERING DNA AND PROTEINS FROM PERUVIAN MUMMIES
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The main objective of this research is to obtain DNA and proteins from 3 Peruvian mummies belonging to two different ancient cultures (Lambayeque and Chachapovas 1.000-1.400 AD) in order to study their molecular genetic relations; this a preliminary report.
From epithelial (foot) tissue of mummies we sectioned with a scalpel portions weighing 0.1-0.3 g. DNA and proteins were extracted and partially purified, using gradual rehydration-DNA-protein extraction with buffer Tris HCL. 50 mM-DNA separation with NaCl/K-Ac. Phenol/Chloroform-Proteins by salting out procedure, as indicated by P. Chimoy (in press). DNA quantified by UV method and molecular weight (M.W.) determined by agarose gel electrophoresis (0.8%) using TBE buffer and visualized with UV lamp 254 nm (Maniatis et al 1982). Proteins by Lowry's method and M.W. by PAGE SDS 10% (MWS-877 Tech.Bull SIGMA).
55.49-86.70 ug of DNA/g. tissue were recovered, observing one band of DNA (15-20 Kb); 25.22-36.47 mg of proteins/g. tissue were recovered and 5 proteins determined (120.105.54.19 and 17 Kd).

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Genetic analysis of apolipoprotein(a) isoform phenotypes. H. Hamaguchi*, A. Nakagawa, K. Kobayashi, S. Kikuchi, H. Yanagi, and T. Annami, Inst. Basic Medical Sciences, Univ. Tsukuba, Tsukuba, Japan

Apolipoprotein(a) (apo(a)) is homologous to plasminogen and is covalently linked to apolipoprotein B, forming lipoprotein(a) (Lp(a)). Plasma Lp(a) concentrations vary more than 100-fold between individuals and high plasma levels of Lp(a) is an independent genetic risk factor for atherosclerotic vascular disease. More than 10 polymorphic apo(a) isoform bands have been distinguished from different individuals by SDS-PAGE and an inverse correlation has been found between the molecular weight of apo(a) and the plasma Lp(a) level.

To develop a sensitive method for the analysis of apo(a) phenotype with high resolution of the isoform bands, we have prepared a monoclonal apo(a) antibody and analyzed apo (a) in plasma lipoprotein fractions of 120 healthy subjects by gradient SDS-PAGE using immuno-blotting with the monoclonal antibody. Radioisotope was not used. A total of 12 different polymorphic bands ranging in the molecular weight more than 520 kD could be resolved, but only 1 or 2 bands were present per individual of all subjects examined. Distribution of plasma Lp(a) concentrations in 120 subjects was similar to that in most Caucasian and a inverse relation between the molecular weight of apo(a) isoforms and plasma concentrations of Lp(a) was observed. The data obtained by a family study including 15 parent-children combinations suggested that the inheritance of apo(a) isoforms is codominant in principle. We are applying this method to the analysis of apo(a) isoform phenotypes in patients with coronary heart disease.

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The glycosylation sites of human lysosomal α-glucosidase. M.M.P. Hermans*, H.A. Wisselaar, M.A. Kroos, B.A. Oostra and A.J.J. Reuser. Dept. of Cell Biology and Genetics, Erasmus University, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands.

Human α-glucosidase is essential for the lysosomal degradation of glycogen. Enzyme deficiency results in the lysosomal storage disorder glycogenosis type II. Glycosylation is an important event in the posttranslational modification of lysosomal α-glucosidase. The cDNA sequence indicates that there are seven potential glycosylation sites. We have eliminated these sites individually by site directed mutagenesis to determine which ones are used. All seven sites were found to be glycosylated. Two were located in the C-terminal trailer peptide which is removed during maturation. At least two of the oligosaccharide side chains of the mature enzyme appeared to be phosphorylated. Elimination of six of the seven sites did not disturb enzyme synthesis or function. But, an effect on the proteolytic processing, the transport and the catalytic function of lysosomal α-glucosidase was observed by removal of the second glycosylation site. Using immuno-electronmicroscopy the enzyme lacking this site appeared to be located only in the endoplasmic reticulum and not in the more distal transport compartments. Evidence for premature degradation was obtained by pulse-chase labeling.

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HPLC of urinary oligosaccharides. P.A. Hommes and M. Varghese. Medical College of Georgia, Augusta, GA.

Carbohydrates can be separated chromatographically on pellicular anion exchange resins operating at high pH and can be detected by pulsed amperometry. These principles have been applied to the separation of urinary oligosaccharides for the diagnosis of glycoprotein degradation disorders, using a Dionex BIOLC HPLC equipped with a pulsed amperometric detector (E_1 =0.05V, T_1=480ms; E_2=0.60V, T_2=300ms; E_3=-0.60V, T_3=200ms). A CarboPac PA1 column and elution with 0.09M NaOH, 0.015M NaAc (15min), a linear gradient to 0.2M NaOH, 0.6M NaAc in 15 min, followed by isocratic elution with this buffer for 10 min. At least 60 compounds can be detected in normal urine with this system, each compound characterized by a highly reproducible retention time (within 5 sec). Each of the glycoprotein degradation disorders investigated (α-mannosidosis, β-mannosidosis, fucosidosis, Sandhoff disease, GM-1 ganglisidosis, sialidosis, galactosialidosis, aspartyl glucosaminuria) as well as sialiluria and glycogen storage disease, type II, yielded a unique pattern with characteristic retention times, facilitating an unambiguous diagnosis. The method is sensitive (10μL of urine required) and fast (40 min).

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Determination of methylmalonyl-CoA mutase activity in human placenta by high performance liquid chromatography. C-J Horng*(1), S-J Wu(2), T-T Liu(2), C-B Sim(1), K-J Hsiao(2)(3), Departments of (1) Psychiatry and (2) Medical Research, Veterans General Hospital-Taipei; (3) Institutes of Genetics, National Yang-Ming Medical College; Taiwan, Republic of China.

A method modified from that of Kikuchi et al.(Clin Chim Acta, 1989;184:307) to determine activity of methylmalonyl-CoA mutase in human placenta was developed. About 150 μg protein of human placenta extract in reaction mixture (50 mM phosphate buffer, pH 7.5) was preincubated with adenosylcobalamin (10 μM) at 30°C for 10 minutes. The reaction was initiated by the addition of DL-methylmalonyl-CoA (0.3 mM), then incubated at 30°C for 5 minutes, and terminated by perchloric acid. The chromatographic seperation of product succinyl-CoA was achieved by a reverse-phase(C-18) column with an isocratic elution (220 mM sodium phosphate, 15% methanol, 0.4% chloroform, pH 4.0) at 35°C and monitored at 254 nm. The coefficient of variance of this method was 3.2 % and 8.7 % for intra- and inter-assay, respectively. The K_m of substrate methylmalonyl-CoA and cofactor adenosylcobalamin was 15 μM and 0.3 respectively. Specific enzyme activity determined in 11 normal placenta ranged from 1.33 to 4.62 mU/mg protein. This method had also been successfully adopted to cultured fibroblasts, which will be in aid of confirming diagnosis of methylmalonic acidemia.

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