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SB-P01

STRUCTURAL EFFECTS OF A MUTATION RESPONSIBLE FOR A GLYCOGENIN RELATED NOVEL GLYCOGEN STORAGE DISEASE

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Glycogen Storage Diseases (GSD) are inherited metabolic disorders of glycogen metabolism. They are classified based on the enzyme deficiency and the affected tissue. The most recently described GSD is due to the deficiency of glycogenin-1, caused by a Thr82Met mutation. Glycogenin is a self-glucosylating protein involved in the initiation of glycogen synthesis. In the presence of UDP-glucose and Mn^{+2} it catalyzes the formation of a short glucose polymer covalently attached to its Tyr194 hydroxyl group. Glycogenin-1 is one of the two human glycogenin isoforms and is mainly expressed in muscle. The second isoform, glycogenin-2, is the liver protein. Human glycogenin-1 displays 93% identity with rabbit skeletal muscle glycogenin, the best studied member of this protein family and the only one whose three dimensional structure has been solved. Thr82 is not only present in human and rabbit glycogenins, it is also conserved in many members of the family. According to the reaction mechanism proposed for the protein, this residue would not be directly involved neither in catalysis nor in substrate binding. To explore the reasons for Thr82Met mutation induced loss of function, we have prepared this mutant form of rabbit glycogenin. We report here the crystal structure of apo- and UDP-glucose/Mn^{+2} bound Thr82Met mutant and the comparison of these results with those of the wild type enzyme.

SB-P02

CONTRIBUTION TO THE KNOWLEDGE OF THE SALIVA PROTEOME AND ITS IMPORTANCE AS A DIAGNOSIS TOOL

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The saliva proteome includes a number of proteins involved in the homeostasis of the oral cavity and there is currently great interest in its utilization as a diagnosis tool. In this work we studied parotid saliva samples from normal children as well as those from children with JIA (Juvenile Idiopathic Arthritis). The 2D-polyacrylamide gel electrophoresis denoted their high complexity degree. Then, two methodologies were used: a) samples were resolved by molecular filtration and fractions analyzed by HPLC-ESI-MS; b) samples were digested by trypsin and then analyzed by nano HPLC-ESI-MS/MS. Results indicate sample degradation prior to saliva analysis and many peptides from parotid saliva proteins, such as alpha amylase and proline rich proteins were found. On the other hand, several proteins were described in normal human saliva for the first time (an actin-binding protein, an alpha enolase, fragments of the immunoglobulin kappa chain, a thioltransferase I, a leucocyte elastase inhibitor, apoliprotein A1). In addition, we found other two proteins only present in JIA samples and never described in normal human saliva. We also reported the finding of a 18460 Da molecular mass present in all JIA samples but absent in normal ones which could be used as a molecular marker for early detection of the disease.

SB-P03

ACTIVATION OF LIMULUS COAGULATION FACTOR G BY SCLEROGLUCAN AS A BIOLOGICAL ACTIVITY MEASUREMENT

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The ability of scleroglucan (ß-1,3-ß-1,6 glucan) conformers to activate Limulus coagulation factor G was evaluated. Studies pursued to get a further insight into the scleroglucan structure-function relationship. Lab-scale produced (EPS I, EPS II, EPSi) and commercial (LSCL) scleroglucans were tested on their ability to activate factor G of the Limulus coagulation cascade (Glucatell Test, Pyrolab). Native triplex conformation was obtained by dissolving scleroglucan (2 pg/mL) in distilled water. Thermally- (150°C, 30 min) and alkali-treated (0•2 N NaOH, 10 min) scleroglucans were also prepared. Single helices were further evaluated at 2 ng/mL and 2 µg/mL. All scleroglucans were endotoxin-free (LAL-Test, Pyrolab). Triplex conformation had stronger ability to activate factor G than single helix, with EPS I and EPS II as the most active polymers. Thermal and alkaline denaturation significantly reduced scleroglucan reactivity, being EPS II the most stable glucan, whilst EPSi and LSCL were the most affected ones (91-99% reduction). Single helix activation ability was significantly dependent on polysaccharide concentration. Scleroglucans from *S. rolfsii* ATCC 201126 (EPS I and EPS II) exhibited marked clotting activity against factor G and were more effective than commercial scleroglucan. Denaturation would lead to lower biological activity and it would be dependent on EPS concentration.

SB-P04

FRET-ASSISTED CONFORMATIONAL STUDIES OF ß-GLUCAN BIOPOLYMERS WITH BIOLOGICAL ACTIVITY

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(1,3)-ß-D-glucans such as Laminarin (Lam) are known to behave as immunopotentiators. Triple-helix is the prevailing conformation in aqueous solution and can be denatured with NaOH as single or partially opened-helices conformers. Which of these conformers is biologically most active or, if complete strands separation actually occurs is unclear. Fluorescence resonance energy transfer (FRET) spectroscopy represents an indirect method to characterize conformational changes of NaOH-treated Lam. FRET phenomenon occurs when a donor excitation energy is transferred to an acceptor over a short distance (10-80 Å). In this work, Lam was derivatized with 1-aminopyrene (AP) as donor (λ 450 nm), and fluorescein-5-isothyocyanate (FITC) as acceptor (λ 520 nm). The chain opening degree was assessed by treating double-labeled Lam (Lam-AP-FITC) with different concentrations of NaOH (0•10 – 1•00 M). FRET results demonstrated that partially opened triple-helix rather than single-helix conformation would be formed upon NaOH treatment of Lam. Increasing degrees of strand opening were associated with increasing concentrations of NaOH. From 0•10 to 0•25 M, FRET showed a significant decrease, involving an actual separation between AP and FITC probes. A conformational change degree between closed triple-helix and partially opened-triple-helix would be more reliable than the complete strands separation.