



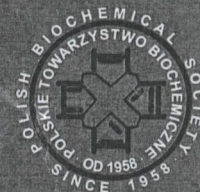
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to the loss of DNA-binding capability. The mutant enzymes with nonclassical phenotype res- mod+ (Nos. 69, Leu 175 Pro and 123, Lys 184 Asn) and one mutant with classical phenotype res- mod- (No. 198, Pro 218 Ser) were biochemically analyzed. We demonstrated that these point mutations influence, though to different extent, the ability of the mutant HsdS subunits to assemble with the HsdM to produce a functional MTase. As a consequence of this, the mutant MTases have reduced DNA binding, as well as the assembly with the HsdR subunits into the functional REase. The central role of HsdS in the assembly of the R-M enzyme has been confirmed and the importance of the junction between the central conserved domain and the target recognition domain (TRD2) clearly illustrated.

P3.2-69

Carboxypeptidases of germinating Triticale seeds (\times Triticosecale Wittm.)

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Endopeptidases and carboxypeptidases play essential role in hydrolysis of storage proteins in cereals. Carboxypeptidases from extracts of dry and germinating Triticale seeds (\times Triticosecale Wittm.) cv. Fidelio rapidly hydrolyzed synthetic substrates with blocked N-terminal group (N-CBZ-dipeptides) at low pH. HPLC fractionation of extracts from endosperm of 3D germinated seeds on SP 8HR cation exchange column revealed the presence of several peaks of carboxypeptidases activity, which differed in substrate specificity. The first one showed the highest activity towards N-CBZ-Phe-Ala and smaller against other hydrophobic substrates (N-CBZ-Phe-Leu and N-CBZ-Phe-Met). Its presence was also observed in embryo of 3D germinated seeds though the enzyme was absent in dry seeds. Molecular weight of the enzyme, estimated with the gel filtration chromatography on Sephadex G-150, was about 148 kDa. The second enzyme that appeared only in endosperm of germinating seeds showed the highest activity towards N-CBZ-Ala-Phe and N-CBZ-Ala-Met and had a low molecular weight. It was not found in an embryo or in the endosperm of dry seeds. Both enzymes resemble, respectively, carboxypeptidases I and III isolated from wheat and barley seeds and synthesized de novo during germination [1, 2]. Additionally, two high peaks of N-CBZ-Phe-Ala activity and molecular weight about 142 kDa (pertaining to the activity of carboxypeptidase II) were observed in seeds after 3 days of germination. Both of them demonstrated the same substrate specificity and were the only, which occur in endosperm of dry seeds. The activity of carboxypeptidases IV and V, with specificity towards substrates that have proline at the penultimate position, was low. These enzymes were found only in endosperm of germinating seeds.

References:

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2. Mikola L. (1983). *Biochim. Biophys. Acta* **747**: 241–252.

P3.2-70

Unfolding and inactivation of prostatic phosphatase during urea and guanidinium chloride denaturation

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The kinetics of the prostatic acid phosphatase (hPAP) denaturation induced by urea and guanidinium hydrochloride has been studied to determine hierarchy and mutual relations in folding of domains as the protein substructures. The inactivation of enzyme, intrinsic fluorescence of tryptophans, the products of limited proteolysis were determined in the course of the protein denaturation. Moreover, to explore properties of kinetic folding intermediates, the accessibility of –SH groups of cysteine side chains, located in different domains of the protein subunits was monitored. The results show that hPAP inactivation in 1.25 M guanidine chloride, pH 7.3, is first-order reaction, with the reaction rate constant $6.6 \times 10^{-2} \text{ min}^{-1}$. The process of enzyme inactivation is accompanied with exposition of –SH groups and strong fluorescence of the product of their reaction with 2-(4'-maleimidoanilino)naphtalene-6-sulfonate (MIANS). The reaction of hPAP inactivation in 3 M urea, pH 7.3, is first-order reaction with the reaction rate constant $1.35 \times 10^{-2} \text{ min}^{-1}$. Complete inactivation of enzyme is accompanied with unmasking of about 50% of free thiol groups that are hidden in the native structure of protein. Identification of products of the limited proteolysis of the partially denatured, inactive protein should indicate fragments that are autonomously folded substructures in the protein.

P3.2-71

Enzymology of CAD and EndoG: two β - β - α -Me-finger nucleases involved in apoptotic DNA fragmentation

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Endonuclease G (EndoG) and the caspase-activated DNase (CAD/DFF40) are two important nucleases involved in different signal transduction pathways leading to apoptotic DNA fragmentation. Both enzymes belong to the ancient superfamily of β - β - α -Me-finger nucleases, implying that, despite their different overall structure and domain organization, they share a common active site fold and display similar catalytic mechanisms as well as comparable DNA binding modes. We have intensively characterized these pro-apoptotic enzymes and based upon our studies propose structural models of their active sites as well as their mode of DNA binding and cleavage. One striking difference between these functionally and mechanistically related enzymes is their substrate specificity. Whereas CAD/DFF40 is a pure DNase, EndoG also cleaves RNA, which could mean that EndoG participates in apoptotic RNA fragmentation. We are therefore investigating the potential of ectopically expressed EndoG to induce cell killing with a focus on its putative *in vivo* RNA fragmentation activity.

P3.2-72

Evaluation of the biochemical activity of new modified steroids as aromatase inhibitors

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Aromatase is the P-450 enzyme responsible for catalyzing the conversion of androgens to estrogens, in different tissues. Its inhibition has shown to be a potential target for the prevention and therapy of some estrogen-dependent diseases, such as endometrial and breast cancer. For this reason, different steroidal aromatase inhibitors have been designed and some have been clinically approved. 4-OHA (4-hydroxyandrostenedione) is one of the example which is a potent inhibitor that blocks estrogen biosynthesis in a competitive manner, but also causes inactivation of the enzyme. Intense research is being carried out in this area with the goal to develop more selective and potent inhibitors. In this respect, our group designed, synthesized, and evaluated biochemical activity of new D-ring modified steroids, in comparison to the known aromatase inhibitor 4-OHA, and determined the IC₅₀. The assays were performed in human placental microsomes, by quantification of the production of ³H₂O release from [1-³H] androstenedione aromatization. Our results showed that inhibition of the enzyme activity by the synthesized steroids, tested at 2 mM, was between 20 and 80% and was dose dependent. The modified steroid that exhibits the highest activity was further characterized by evaluating the type of inhibition. Our findings demonstrated that the inhibitor with a δ -lactone D-ring, replacing the usual cyclopentanone ring, was about four times less active *in vitro*, using human placental microsomes. In summary, our results suggest that this modification gives no benefit to the inhibitory activity of newly synthesized compounds, permitting to conclude that the binding site of aromatase, corresponding to the D-ring steroid region, is very restrictive.

P3.2-73

Elucidation of the mechanism for successive methyl group transfers by set domain-containing protein methyltransferases

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Protein methylase III enzymes are a unique class of post-translational processing enzymes responsible for the mono-, di-, or tri-methylation of the ϵ -amino groups

cyclosporin A and FK506 inhibit rat glioma cell growth and induce apoptosis. We demonstrated that NFATc proteins are present in rat C6 glioma cells and to less extent in immature astrocytes, but are not expressed in nontransformed, adult astrocytes. Our aim was to determine the role of NFAT pathway in glioma cell function. We used C6 glioma cells stably expressing SPRIET (a short inhibitory peptide that disrupts a critical calcineurin - NFAT interaction) and SPAIAA (a control peptide). An activity of NFAT pathway was tested using NFAT - driven luciferase reporter plasmid. The results confirmed that SPRIET transfectants are unable to upregulate NFAT-dependent expression upon treatment with TPA/Ionomycin. We thus assessed morphological differences between transfectants cultured in regular and serum - free medium and did cell proliferation studies by flow cytometry. Using MTT assay we studied cell's response to cytotoxic insults. Since NFAT has been reported to bind and regulate expression of cytokines and early immune response genes, we hypothesized that transfectants lacking NFAT functional pathway can differ in the profile of secreted cytokines. RT-PCR analysis revealed differences in the selected cytokine expression. This work was supported by grant PBZ-MIN-001/P05/2002 from the State Committee for Scientific Research (Poland).

P4.1-51

Effect of ROCK pathway on morphology and cytoskeleton organization in C6 glioma cells

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Small GTPase Rho and one of its effectors Rho-dependent kinase (ROCK) are known to regulate a variety of cellular responses via controlling organization of actin cytoskeleton as well as actomyosin interaction. We examined the effects of blocking ROCK by its specific inhibitor Y-27632 on cell morphology and the cytoskeleton arrangement in C6 glioma. The cells were incubated in the presence of 100 μ M Y-27632 for about 30 min. Double-staining of ROCK and F-actin in untreated cells revealed colocalization of ROCK with F-actin both in the cortical region and along stress fibers; ROCK was also found to be dispersed throughout the cytoplasm. Nonmuscle myosin II was localized in the form of punctate staining, corresponding to myosin oligomers, concentrated in the cell body, particular in the perinuclear area and along actin stress fibers. Y-27632 induced drastic and irreversible changes in cell morphology as cell bodies of treated cells rounded up and protruded elongated projections; numerous blebs were found around the entire cell surface. We observed disappearance of stress fibers, F-actin was present mainly in the cortical region where it colocalized both with myosin II and ROCK. Myosin and actin also colocalized in the blebs. These results indicate that Rho/ROCK pathway plays the essential role in actin-cytoskeleton remodeling in C6 glioma cells. The molecular mechanism of ROCK function in these tumor cells is to be revealed but the described data imply that it certainly affects actomyosin contractility.

P4.1-52

Expression of vascular endothelial growth factor (VEGF) in rat normal pregnancy and spontaneous fetal-resorption

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In response to the implanting blastocyst, endometrial stromal cells proliferate and differentiate into decidual cells forming the decidua. After reaching maximum development, the decidua undergoes programmed cell death. In rat pregnancy although the majority of fetal-placental units develop successfully until term, a minority fail during early pregnancy and undergo "spontaneous resorption" without affecting adjacent units. The mechanisms underlying this natural pregnancy loss are unknown. Angiogenesis is essential for normal placentation and disruption of this process may lead to pathological disorders. In this study we examined the spatial and temporal patterns of expression of the angiogenic growth factor VEGF, in rat maternal uterine tissues throughout normal gestation and in the fetal-placental units undergoing

spontaneous resorption. The immunohistochemical analysis revealed an increase in VEGF expression reaching its maximum on day 16, declining thereafter. From day 8 till 11 immunoreactivity was found in the antimesometrial decidua. On day 12 the expression moved to the mesometrial decidua paralleling antimesometrial regression. On day 16, expression was localized in the thin layer of the regressing decidua and in the NK cells of decidua and metrial gland (MG). On this day the resorpted units presented a decrease in expression in the MG possibly due to a smaller number of NK cells expressing VEGF. Our results suggest that VEGF may exert an important role within tissue remodeling of maternal decidua. Spontaneous foetal-resorption was not associated with altered expression of VEGF in this tissue. However, the alterations observed in MG may indicate localized disturbances of the angiogenic process that deserve further evaluation.

P4.1-53

Regulation of betaine: homocysteine methyltransferase by insulin, glucocorticoids and diabetes mellitus

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Elevated plasma homocysteine has been identified as a risk factor for cardiovascular disease and Alzheimer's disease. We are exploring the role of a number of hormones in determining plasma homocysteine levels. Our studies on various models of diabetes have shown that diabetic animals with no clinical signs of renal dysfunction have lower plasma homocysteine levels than controls or than insulin-treated diabetic animals. Homocysteine is formed by S-adenosylmethionine-dependent methylation reactions and removed by remethylation to methionine by either methionine synthase or betaine:homocysteine methyltransferase (BHMT) or by transsulfuration to cysteine via cystathionine- β -synthase (CBS). We have previously shown that CBS synthesis and activity are downregulated by insulin and are upregulated by glucocorticoids. Methionine synthase is unaffected by diabetes or by insulin treatment. In the current study, we determined BHMT activity and mRNA levels in livers of streptozotocin-diabetic rats and in Zucker Diabetic Fatty (ZDF) rats. In both cases, BHMT activity and mRNA levels were increased by diabetes. In the rat hepatoma cell line, 4HIIIE, insulin decreased the level of BHMT mRNA in a dose-dependent manner and decreased BHMT mRNA synthesis as determined by PCR-based nuclear run-on assays. In the same cell line, glucocorticoids (triamcinolone) increased the level and synthesis rate of BHMT mRNA. Thus the decreased plasma homocysteine in various models of diabetes in animals could be due to some combination of increased transsulfuration of homocysteine to cysteine and increased remethylation of homocysteine to methionine by BHMT. (Supported by Canadian Diabetes Association).

P4.1-54

Effect of tumor necrosis factor α and interferon γ in primary decidual cell culture

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During gestation the uterus undergoes a series of programmed morphologic and physiologic changes from implantation till the mature placenta. In the rat, in response to blastocyst implantation, the stromal cells of the endometrium proliferate and differentiate into decidual cells, forming the decidua. After reaching its maximum development, the decidua undergoes regression to form the maternal component of the placental bed. Recent results obtained in our laboratory indicate that during pregnancy, apoptosis is programmed temporally and spatially with different rates and is associated with a shift in the expression of the anti and pro-apoptotic markers, Bcl-2/Bax. However, the regulatory mechanisms underlying apoptosis in the decidua are, as yet, not clarified. In order to investigate the molecular regulatory mechanisms involved in decidual involution, primary decidual cell cultures were employed. In this system, cytokines present and synthesized within the uterine environment during