PURIFICATION OF PROTEASOMES FROM SALMONID FISH SPERM AND THEIR LOCALIZATION ALONG SPERM FLAGELLA.

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Motility of demembranated sperm in salmonid fish is inhibited by chymotrypsin inhibitors in an ATP-dependent manner. We purified chymotrypsin-like proteases from chum salmon sperm, with the molecular masses of 950 and 650 kD. Some enzymatic properties and molecular shape of the 650 kD protease showed that this protease was a multicatalytic proteinase (proteasome) as is well known to participate in ATP-dependent degradation of ubiquitinated proteins. We prepared polyclonal antibody against purified 650 kD proteasome. This antibody recognized mainly the 29 and 28 kD subunits of proteasome. Using immuno-fluorescent microscopy, we examined the subcellular localization of proteasomes in sperm. The result showed that proteasomes are located predominantly in sperm flagella. Interestingly, the anti-proteasome antibody did not stain overall portion of sperm tail but showed patches or somewhat periodical staining patterns along sperm flagellum. These results suggest that activation of proteasomes at regular intervals on sperm flagellum causes ATP-dependent conversion of microtubule sliding to flagellar bending, resulting in the regulation of sperm motility.

PROTEIN PHOSPHORYLATION IN POLYMORPHONUCLEAR LEUCOCYTES RELATED TO THE SIGNAL TRANSDUCTION OF CHEMOATTRACTANT STIMULATION

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Human polumorphonuclear leucocytes (PMN) are known to be activated by various kind of chemoattractants, LUCT/IL-8, leucotriene B_4 or FMLP, lead to migration, phagocytosis or O_2^- production. To elucidate the molecular mechanism of signal transduction, phosphorylated proteins are analyzed by two-dimensional electrophoresis and autoradiography, after the ^{32}P-labelling of intact PMN and stimulation with FMLP . Protein subunits having molecular weight of 82, 66, 64, 58, 55 and 50 kDa were able to be detected, the marked phosphorylation was observed with 64 kDa proteins. One of the 64 kDa proteins are revealed to be phosphoglucomutase, this phosphorylation was stimulated in presence of a micromolar level of glucose, by a mechanism including hexokinase and substitution of ^{32}P-phosphate from glucose-6-phosphate to active site seine. The other phosphorylatable 64kD protein (p64) was detected after FMLP stimulation, having a isoelectric point (pI=5.3) different from phosphoglucomutase. The pI shifted after phosphorylation from 5.3 to more acidic side forming pp64. The FMLP-stimulated phosphorylation was time-dependent and saturated within 5 min., the maximum stimulation was achieved with 10 nM FMLP. Phosphoamino acid analysis of the pp64 revealed the phosphorylation of the serine residue. Staurosporine (100 nM) and W-7 (100 mM) significantly inhibited the phosphorylation, H-7 slightly inhibited, H-8 and herbimycin did not affect. These data suggested that protein kinase C and calmodulin like protein(s) are concerned. From the purification studies of p64 and by amino acid analysis, p64 was identified as l-plastin, one of the leucocyte specific proteins. Phosphorylation of this may play a role in cytoskeletal reorganization of PMN.

MITOSIS-SPECIFIC PHOSPHORYLATION OF MICROTUBULE ASSOCIATAED PROTEINS

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Mitotic Hela cell extracts exhibited much higher kinase activities to phosphorylate brain microtubule associated proteins (MAP2 and MAP1c) than the extracts from non-mitotic cells. This MAP2/MAP1c kinase activity appears to be distinct from A-kinase, cGMP-dependent protein kinase, C-kinase or Ca^{2+}-calmodulin dependent protein kinase II. Purified cdc2 kinase ($p34^{cdc2}$-cyclin B complex) phosphorylated MAP2 and MAP1c.

INDUCTION OF AMOEBOID MOVEMENTS IN THE SEA URCHIN EGG BY PROTEIN PHOSPHORYLATION.

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Calyculin A, an inhibitor of protein phosphatases type 1 and type 2A, induces cleavage-like changes in the unfertilized sea urchin egg (Tosuji et al., Proc. Nat. Acad. Sci. USA, in press). It induces formation of protrusion or amoeboid movements in the fertilized eggs. We recently found that calyculin A induces the amoeboid movement also in the unfertilized eggs at appropriate concentrations. These eggs usually did not cleave, but cleavaed by the addition of IBMX or TPA. TPA alone could induce the movements in the fertilized eggs. Tautomycin, which has been known to be another inhibitor of protein phosphatases type 1 and type 2A (Hori, M. et al., FEBS Lett., 285: 145-148, 1991) also induced the movements in the fertilized sea urchin eggs. These results strongly suggest that protein phosphorylation is involved in the cytoskeletal organization in the sea urchin egg.