211 REGULATION OF ENZYMES BY LIGAND INDUCED CHANGE IN POLYMERIZATION.

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Many enzymes readily change their aggregation state, by dissociation or association of subunits. This constitutes a mechanism for regulation, if the different molecular weight species vary in their specific activity, and if the equilibrium between these species is readily perturbed by physiological effectors. Our studies show that several enzymes involved in the synthesis or degradation of pyrimidines exhibit such regulation: The multifunctional protein UMP synthase (EC 2.4.2.10 + EC 4.1.1.23), and uridine kinase (EC 2.7.1.48) from Ehrlich ascites cells, and N-carbamoyl-β-alanine amidohydrolase (EC 3.5.1.6) from rat liver. Generally, substrates are positive effectors, while immediate or more distant end products are negative effectors.

UMP synthase shows only positive regulation: The 50,000 dalton monomer is inactive; effectors result in association to the active dimer. Uridine kinase shows negative regulation: the native enzyme exists as polymers of 4 or more subunits, and CTP or UTP produce dissociation to the 31,000 dalton subunit and loss of activity. NCβA amidohydrolase shows bimodal regulation: the native enzyme has an Mr of 235,000; the substrate produces association to 470,000 daltons; the product, β-alanine, causes dissociation to 120,000 daltons.

Ligand induced changes in polymerization may be a general mechanism for regulating enzyme activity.

212 THE MECHANISM OF INSULIN-INDUCED INCREASE OF THE RATE OF DE NOVO PURINE BIOSYNTHESIS IN PRIMARY CULTURED RAT HEPATOCYTES

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The de novo purine biosynthesis was studied 8 hours after the administration of insulin. The rate of de novo purine biosynthesis assayed by [^{14}C]formate incorporation started to increase at the insulin concentration of 10^{-10}M and reached the plateau at 10^{-7}M or above with its level 2.8 folds higher than control. 5-phosphoribosyl 1-pyrophosphate(PRPP) availability assayed by [^{14}C]adenine incorporation increased steeply and reached the plateau with its level 2.3 folds higher than control at 10^{-9}M and stayed at this level in the higher concentration. By the addition of Actinomycin D(1μg/ml) or cycloheximide(1μg/ml) increased rate of de novo purine biosynthesis and increased PRPP availability decreased to the control level. Amidophosphoribosyltransferase(ATase) activity per unit amount of protein or DNA increased 1.5 to 1.8 folds with increased Vmax and unchanged Km for PRPP. ATP concentration increased by 27% which was counterbalanced with decreased concentrations of AMP(73%), ADP(69%), GMP(73%) and GDP(79%) concentration. Results of these studies suggest that insulin increases the rate of de novo purine biosynthesis by increasing ATase activity and PRPP availability.

213 THE EFFECTS OF URIDINE AND THYMIDINE ON THE DEGRADATION OF 5-FLUOROURACIL (5-FU), URACIL AND THYMINE BY RAT LIVER DIHYDROPYRIMIDINE DEHYDROGENASE (DPD).

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The effects of various nucleosides and nucleotides on the activity of DPD partially purified from rat liver was studied using 5-FU, uracil and thymine as substrates. 5-FU was found to have a Km similar to those of uracil and thymine. DPD showed the highest specific activity when utilizing 5-FU as substrate (Table).

	5-FU (N)†	Uracil (N)	Thymine (N)
Km*	3.5 ± 0.4§ (13)	2.3 ± 0.3 (11)	2.2 ± 0.3 (10)
specific activity Δ	0.82± 0.04 (18)	0.68± 0.05(20)	0.56± 0.04 (14)

*calculated Km in μM, Δnmols/min/mg protein, §mean ± S.E., †number of individual liver preparations.

Uridine was found to be a potent non-competitive inhibitor of DPD for all three substrates. Thymidine is much less potent as an inhibitor. Compared to 5-FU and uracil, thymine reduction is the most susceptible to inhibition by uridine and thymidine (Table).

Inhibitor	5-FU*	Uracil	Thymine
uridine	0.71	0.62	0.16
thymidine	24	41	8

*Ki expressed as μM

Uridine may enhance 5-FU cytotoxicity more than thymidine.

214 THYMIDINE INCORPORATION IN NUCLEOSIDE TRANSPORT DEFICIENT T LYMPHOMA CELLS.

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Nucleoside transport deficiency in mammalian cells is generally associated with an inability to transport all nucleosides and an inability to bind or be affected by p-nitrobenzylthioinosine (NBMPR), a potent inhibitor of nucleoside transport. Among nucleoside transport-deficient (NT$^-$) S49 T lymphoma clones, however, the ability to take up and respond to the physiological effects of thymidine varied considerably. The NT$^-$ clone, AE_1, was almost as capable of incorporating thymidine into TTP as the wildtype parent. This incorporation of thymidine into TTP by AE_1 cells and a large percentage of thymidine incorporation into TTP by wildtype cells were unresponsive to NBMPR. Thus, thymidine uptake in NT$^-$ cells can be uncoupled genetically or pharmacologically in a specific manner from the uptake of other nucleosides. Consequently, AE_1 cells were just as sensitive as wildtype cells to thymidine toxicity but were resistant to two thymidine analogs, BrdUrd and FdUrd. Moreover, the growth inhibitory effects of thymidine were ameliorated to a much greater extent in wildtype cells than in AE_1 cells by either NBMPR or deoxycytidine. Interestingly, however, AE_1 cells could not grow in hypoxanthine-amethopterin-thymidine containing medium. Initial rates of thymidine entry indicated that AE_1 cells and NBMPR-treated wildtype cells transported thymidine at measurable rates. This residual thymidine transport capability can account for the phenotype of the NT$^-$ cells toward thymidine.

215 REGULATION OF PURINE BIOSYNTHESIS IN G1 PHASE ARRESTED MAMMALIAN CELLS.

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The effects of G1 phase growth arrest on purine biosynthesis were studied in cultured S49 T lymphoma cells. Incubations of wildtype cells for 18 hr with dibutyryl cyclic AMP, which induces G1 arrest, reduced the rates of purine biosynthesis by 95%. Time course and concentration dependence studies indicated that the decrease in rates of purine biosynthesis correlated with the extent of G1 phase arrest. Similar studies with somatic cell mutants deficient in some component of cyclic AMP action or metabolism indicated that the depression in purine synthetic rates required G1 arrest and did not result from cell death. Rates of RNA and DNA synthesis were also markedly diminished in the growth arrested cells. Measurements of purine synthesis in the presence of azaserine indicated that there was an early block in purine biosynthesis prior to the formation of phosphoribosylformylglycinamide. Additionally, the activities of adenylosuccinate synthetase and IMP dehydrogenase were diminished in G1 arrested cells. The levels of all controlling enzymes, substrates, and cofactors, however, were similar in cycling and G1 arrested cells. Despite diminished rates of purine biosynthesis, the levels of intracellular nucleotides in G1 cells were not reduced. These results suggest that perturbations in the consumption of nucleotides via inhibition of nucleic acid synthesis have profound effects on the purine pathway and indicate the importance of feedback inhibition by nucleotides in the regulation of purine synthesis in situ.

216 MYOADENYLATE DEAMINASE DEFICIENCY: DIAGNOSIS BY FOREARM ISCHEMIC EXERCISE TESTING AND PLASMA PURINE MEASUREMENTS.

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Quantitation of lactate and ammonia (NH_3) production after forearm ischemic exercise (FIE) has been proposed as a screening test for myoadenylate deaminase (MAD) deficiency. Plasma lactate, NH_3 and purines (adenosine inosine, hypoxanthine, xanthine) were determined 1, 3, 5 and 10 minutes after 90 seconds of FIE and correlated with exercise performance (Σ kg x sec), measured with a grip dynamometer, strain gauge and strip recorder.

In 13 normals lactate and NH_3 production correlated with exercise performance (r=0.76 and 0.72, respectively). Three patients with histochemically-defined MAD deficiency failed to generate NH_3 despite increasing lactate and adequate performance. Retesting normals at submaximal performance produced lactate and NH_3 generation patterns indistinguishable from those of the 3 patients. Normal subjects even with submaximal performance increased plasma purines from 10 to 38 μM after FIE. The MAD deficient patients raised their purine levels only 0-7 μM.

These data demonstrate that submaximal exercise efforts by normals can give FIE test results indistinguisable from those of MAD deficient individuals if only lactate and NH_3 are assessed and plasma purine measurements after FIE testing are effective in identifying the deficiency state. These results support the hypothesis that disordered purine metabolism occurs in patients with MAD deficiency.