85 COMPARISON OF E.COLI ADENINE PHOSPHORIBOSYLTRANSFERASE AND OTHER PHOSPHORIBOSYLTRANSFERASES

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The gene for E. coli adenine phosphoribosyltransferase (apt) has been cloned and sequenced. The deduced amino acid sequence has considerable homology with amino acid sequences for other phosphoribosyltranseferases (the E. coli phosphoribosyltransferases which utilize glutamine, guanine, or orotate and the mammalian hypoxanthine-guanine and mouse adenine phosphoribosyltransferases). With the exception of the Salmonella typhurium ATP-phosphoribosyltransferase, they all have a common core with a consensus sequence of VAL-leu-x-VAL-ASP- ASP -leu-x-x-THR-GLY-gly- THR . The amino acids in capitals are present in at least 5 of 6 sequences, with the underlined amino acids being invariant. Other specified amino acids are present in at least 3 of 6 sequences. The purine phosphoribosyltransferases also have a region with either ser-tyr or thr-tyr which is probably involved in binding the purine base. The E.coli apt gene has a relatively long leader sequence which can base pair with the coding sequence, and may play a role in regulation. The gene is followed by sequences which may act as transcription terminators.

88 INCREASED INOSINATE DEHYDROGENASE ACTIVITY IN MYCOPHENOLIC ACID RESISTANT NEUROBLASTOMA CELLS

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Mouse neuroblastoma cells, NB, have been grown in increasing concentrations of mycophenolic acid for greater than 80 passages. A mycophenolic acid resistant neuroblastoma cell line (NB-MyR) has been isolated which is 1000-fold more resistant to mycophenolic acid than NB cells. Fifty percent growth inhibition was caused by 1.6×10^{-7} M and 1.65×10^{-4} M mycophenolic acid for NB and NB-MyR cells respectively. In crude cell lysates the apparant inhibition constant for mycophenolic acid with inosinate dehydrogenase was 6×10^{-8} M and 14×10^{-8} M for NB and NB-MyR cells respectively. Thus the ki for mycophenolic acid was essentially unchanged. The specific activity of inosinate dehydrogenase was 4.9 ± 1.4 and 26.6 ± 2.5 nmole/min/mg protein for NB and NB-MyR cells respectively. The 5.4-fold increase of inosinate dehydrogenase activity in the NB-MyR line may account in part for the increased resistance to mycophenolic acid. Preliminary findings also suggest a 3-fold increase in guanylate synthetase activity for NB-MyR cells as compared to NB cells.

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86 HEMOLYTIC ANEMIA AND LOW RED CELL ATP/dATP RATIO IN GENETIC ADENOSINE DEAMINASE DEFICIENCY.

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Marked depletion of ATP and hemolysis have been reported to accompany dATP accumulation in erythrocytes of patients undergoing treatment with the ADA inhibitor deoxycoformycin (dCF), but not in patients with inherited ADA deficiency. It has therefore been unclear whether ATP depletion and hemolysis were caused by ADA inhibition per se, or by unrelated effects of phosphorylated derivatives of dCF. We have studied a 9 month old ADA deficient girl who at diagnosis had a heinz body positive hemolytic anemia as well as immune deficiency. Her RBC's underwent spontaneous lysis in vitro, which was not corrected by glucose or enhanced by osmotic or pH stress. Known causes of anemia were excluded. ATP, measured on 4 occasions, was 0.69 +/- 0.14 umol/ml RBC's (range=0.5-0.8) (controls=1.4 +/- 0.3). dATP was 1.24 +/- 0.22 umol/ml RBC's (normally <0.002); the ratio of ATP:dATP was 0.46-0.67. We have previously described a mechanism by which dATP accumulation can induce marked ATP catabolism (Bagnara and Hershfield, PNAS 79:2637, 1982). Thus, ADA deficiency, like pyruvate kinase deficiency and massively increased erthrocyte ADA activity, may be an inborn error of metabolism that can lead to hemolysis by causing ATP depletion. However, in most cases some compensatory mechanism must operate to prevent ATP depletion as marked as in the patient we have described.

89 REGULATION OF DEOXYADENOSINE AND NUCLEOSIDE ANALOG PHOSPHORYLATION BY HUMAN ADENOSINE KINASE.

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The phosphorylation of dadenosine and nucleoside analogs is important in adenosine deaminase deficiency and for specific anticancer and antiviral drugs. We examined the regulation of nucleoside phosphorylation by adenosine kinase purified 4000-fold (2.1 µmol/min/mg). The Km values of dadenosine and ATP were 135 µM and 4 µM, respectively. Potassium and magnesium are absolute requirements for dadenosine phosphorylation and 150 mM potassium and 5 mM $MgCl_2$ are critical for linear kinetics. At 0.4 mM $MgCl_2$ in excess of ATP levels, the Km for dadenosine increased 10-fold. ADP is a competitive inhibitor with a Ki of 13 µM with variable $MgATP^{2-}$, while it is a mixed inhibitor with a Ki and Ki' of 600 µM and 92 µM, respectively, when dadenosine is variable. AMP is a mixed inhibitor with Ki and Ki' of 180 µM and 20 µM, respectively, with variable dadenosine. Adenosine kinase phosphorylates adenine arabinoside (ara-A) with an apparent Km value of 1 mM. The Km values for 6-methylmercaptopurine riboside and 5-iodotubercidin were estimated to be 7 µM and 3 nM, respectively. These data indicate that dadenosine phosphorylation is regulated by its Km, and levels of Mg, ADP, and AMP. The high Km values for phosphorylation of dadenosine and ara-A suggest that adenosine kinase may have a limited role for phosphorylation of these nucleosides in vivo.

87 THE STABILIZATION OF ASCORBIC ACID BY URIC ACID.

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In recent years, several antioxidant activities of uric acid have been described (PNAS 78:6858, 1981; FEBS LETT. 174:147, 1984). The high reactivity of urate with singlet oxygen, hydroxyl radicals and various organic peroxides suggest that there may be important biological functions associated with such scavenging activity. We now report that, in addition to the foregoing capacities, uric acid increases the stability of ascorbic acid in serum about five-fold. A striking feature of this effect is the absence of measurable uric acid disappearnace despite the marked inhibition of ascorbic acid oxidation. Thus, the stabilizing effect appears distinct from previously described stochiometric scavenging functions. Uric acid prevents the oxidation of ascorbic acid by an as yet undefined mechanism which does not involve its extensive oxidation to allantoin or other products. This stabilizing effect may have important consequences for the conservation of ascorbic acid in human blood and other biological fluids. The evolutionary loss of the ability to synthesize ascorbic acid may have been accompanied by a complementary loss of uricase. The latter mutation would insure levels of uric acid sufficiently high to stabilize ascorbic acid, derived from dietary sources, for utilization at intracellular sites.

90 EXPRESSION OF MURINE ADA cDNA IN E. COLI AND MAMMALIAN CELLS.

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Adenosine deaminase (ADA) has been shown by genetic and biochemical evidence to be essential for the development of the immune system. In order to study ADA gene structure, regulation and expression, we have isolated mouse cell lines which contain amplified copies of ADA genes. In some cell lines ADA accounts for more than 75% of the soluble protein, representing an increase of 11,400 fold over parental cells. A cDNA library was constructed in pBR322 using an amplified cell line. Functional mouse ADA cDNA clones were isolated by genetic complementation of ADA-deficient E. coli. Analysis of plasmids containing functional ADA cDNA sequences suggested that ADA expression resulted mainly from β-lactamase/ADA fusion proteins. The nucleotide sequence of a 1.65 kb insert was determined and found to contain a 1.056 kb open-reading frame (ORF). When this ORF was inserted into a bacterial expression vector, only a single band of murine ADA was detected upon starch gel analysis. This ORF was placed in a mammalian expression vector and introduced into COS cells where a high level of authentic murine ADA was obtained. A variety of rodent cell lines have been transformed using this vector. Helper-free preparations of retroviruses have been prepared which are capable of transducing functional ADA cDNA into cultured mammalian cells. These retroviral vectors should allow us to introduce the gene into hematopoietic stem cells.