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Phosphorylation of 9-(2-Phosphonomethoxyethyl)adenine and 9-(S)-(3-Hydroxy--2-phosphonomethoxypropyl)adenine by AMP(dAMP) Kinase from L-1210 Cells.

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Acyclic nucleotide analogues 9-(2-phosphonomethoxyethyl)adenine (PMEA) and 9-(S)-(3-hydroxy-2-phosphonomethoxypropyl)adenine (HPMPA) which display potent antiviral activity are transformed in the cells to their mono- and diphosphoryl derivatives. We purified the enzyme from the cell-free extract of mouse leukemic cells L-1210 which is capable of the two-step phosphorylation of PMEA and HPMPA to their diphosphoryl derivatives and found that it copurifies with AMP(dAMP) kinase activity. It has the molecular weight of 68 000 and is most likely composed of two subunits of 40 000 and 29 000; it differs thus from AMP kinases which occur regularly as monomers with MW between 23 000 and 32 000. The best substrates of this enzyme are AMP, ADP and dAMP. Phosphorylation of HPMPA and PMEA proceeded with 210 and 1400 times lower velocity than the reaction with AMP. Isoelectric point: 7.1 . ATP could not be substituted as phosphate donor by other nucleoside 5'-triphosphates or by creatine phosphate. The enzyme also phosphorylates monophosphates of PMEA and HPMPA to their diphosphates regardless of the presence or absence of ATP--regenerating system. The purified enzyme phosphorylates solely the (S)--enantiomers of HPMPA and its 3'-fluoro analogue FPMPA, the (R)-enantiomers are intact. The phosphorylation of cytosine analogue HPMPC is not mediated by this enzyme.

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Quantitation of Acyclic Adenine Nucleoside Phosphonate Concentrations in Human Plasma Using High-Performance Liquid Chromatography and Fluorescence Detection

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9-(2-Phosphonylmethoxyethyl)adenine (PMEA), (*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine (HPMPA) and (*R,S*)-9-(3-fluoro-2-phosphonylmethoxypropyl)adenine (FPMPA) are antiviral agents with selective activity against DNA viruses and/or retroviruses. A highly sensitive method has been developed to determine levels of these compounds in human plasma. Plasma samples containing PMEA, HPMPA or FPMPA were treated with chloroacetaldehyde yielding the corresponding highly fluorescent $1,N^6$-ethenoadenine derivatives. Chromatographical separation combined with excitation at 254 nm and fluorescence detection at 425 nm allowed quantitative detection of PMEA, HPMPA and FPMPA within a concentration range of 0.08 to 1200 µg/mL. This method proved useful in accurately determining low PMEA concentrations in the serum of PMEA-treated monkeys and cats. The assay may be applied in the quantitation of PMEA, HPMPA and FPMPA concentrations in plasma and urine of humans treated with either of these drugs. The assay has also proved useful in monitoring intracellular uptake of PMEA in cultured lymphocytes, and thus could be applicable to the quantitation of intracellular concentrations of PMEA (and its metabolites) in lymphocytes (or other cells) in patients treated with PMEA.