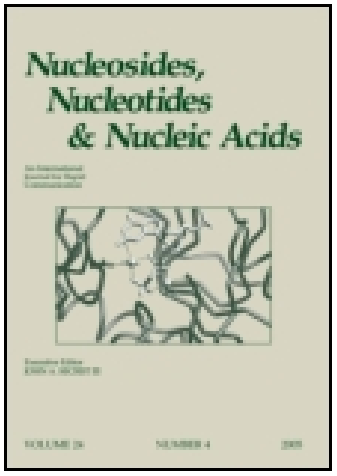

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Pyrimidine Nucleotidases/ Phosphotransferases from Human Erythrocyte

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PYRIMIDINE NUCLEOTIDASES / PHOSPHOTRANSFERASES FROM HUMAN ERYTHROCYTE

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ABSTRACT: Two cytoplasmic pyrimidine 5'-nucleotidase have been purified from human erythrocytes to homogeneity and partially characterized. The two enzymes, indicated as PN-I and PN-II, preferentially hydrolyse pyrimidine 5'-monophosphates and 3'-monophosphates, respectively. The kinetic analysis demonstrate that pyrimidine 5'-nucleotidases, in the presence of suitable nucleoside substrates, can operate as phosphotransferases by transferring phosphate to various nucleoside acceptors, including nucleoside analogues known as important drugs widely used in chemotherapy.

Erythrocyte pyrimidine 5'-nucleotidases specifically catalyze the dephosphorylation of various pyrimidine nucleoside monophosphates to their respective nucleosides. Two activities, called PN-I and PN-II, were identified in the soluble fraction of human erythrocytes on the basis of their different substrate specificities[1]. Nucleotidase and phosphotransferase activities were measured by a HPLC-based assay, as previously described[2], with slight modifications. Alternately the enzyme activity was assayed by measuring the amount of phosphate released. For the measurement of the phosphotransferase activity the incubation mixture contained: 100 mM Tris-HCl pH 7.4, 10 mM $MgCl_2$, 0.04 mg/ml BSA, 5 μM - 5 mM nucleoside monophosphate donor, 1-40 mM nucleoside acceptor, and an appropriate amount of enzyme to ensure initial rate conditions. After the incubation of 30 min at 37°C, the reaction was terminated by the addition of 0.4M $HClO_4$ and the mixture, neutralized with 1M K_2CO_3, was analyzed by HPLC, using a C-18 reversed phase (250 x 4.6 mm) column. After equilibration with 0.1 M KH_2PO_4, pH 6.0 buffer, the substrates and the products were separated by a linear gradient of methanol ranging from 0 to 20% in the same buffer.

The two cytoplasmic forms of pyrimidine 5'-nucleotidase have been purified from human erythrocytes to apparent homogeneity and partially characterized in our laboratory[3]. PN-I and PN-II preferentially hydrolyse pyrimidine 5'-monophosphates and 3'-monophosphates, respectively. It has been also found that PN-I and PN-II are active on nucleoside monophosphate analogues, known as important drugs, like 3'-azido-3'-deoxy-thymidine-5'-monophosphate (AZT-MP), cytosine-ß-D-arabinofuranoside-5'-monophosphate (Ara-CMP), and 5-fluoro-deoxyuridine-5'-monophosphate (5'-FdUMP). In view of the involvement of the nucleotidases catalyzed reactions in the release of erythrocyte-encapsulated pyrimidine pro-drugs, the knowledge of their kinetic and regulatory properties might contribute to modulate drugs delivery rate[4]. The kinetics of PN-I and PN-II were examined by using a broad range of substrates. The homogeneous PN-I catalyzes the dephosphorylation of several pyrimidine nucleoside monophosphates in the order: 5'UMP > 5'CMP > 5'dCMP > 5'dTMP > > 5'dUMP > 5'AZT-MP; p-nitrophenylphosphate was a very poor substrate. The homogeneous PN-II catalyzes the dephosphorylation of several pyrimidine nucleoside monophosphates in the order: 3'dTMP > 3'dUMP > 3'UMP > 5'FdUMP > 2'UMP > 3'GMP > 5'dIMP > 5'UMP > 3'dGMP > 5'IMP > 5'UMP > 2'GMP; p-nitrophenylphosphate was also in this case a very poor substrate. Among a large variety of compounds tested as possible effectors of the two enzymatic activities, only the reaction products exerted an inhibitory action. Kinetic analysis showed that phosphate and nucleosides are competitive and non-competitive inhibitors, respectively, suggesting an Ordered Uni-Bi mechanism for the reaction.

The two enzymes, in the presence of suitable nucleoside substrates, can also act as phosphotransferases, catalyzing the transfer of the phosphate moiety from a nucleoside monophosphate donor to a nucleoside acceptor. The evaluation of the kinetic analysis of the phosphotransferase activity is not straightforward, since the enzymatic proteins simultaneously catalyze both hydrolytic and phosphotransferasic reactions. The results obtained are consistent both with a Ping-Pong and an Odered Bi Bi mechanism. PN-I phosphotransferase activity revealed higher affinity for oxy- nucleosides with respect to deoxy- nucleosides, whereas the contrary seems to be true for PN-II phosphotransferase associated activity. Among various pyrimidine nucleoside acceptors tested, also 3'-azido-

3'-deoxythymidine (AZT), cytosine-ß-D-arabinofuranoside (AraC) and 5-fluoro-2'-deoxyuridine (5FdUrd) were phosphorylated. These results show for the first time that soluble pyrimidine nucleotidases are endowed with pyrimidine-specific phosphotransferase activity. These observations suggest that the two cytoplasmic pyrimidine 5'-nucleotidases operate as interconverting activities, capable of transferring the phosphate from the pyrimidine nucleoside monophosphate donor(s) to various nucleoside acceptors, including important drugs like, pyrimidine analogues widely used in chemotherapy.

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