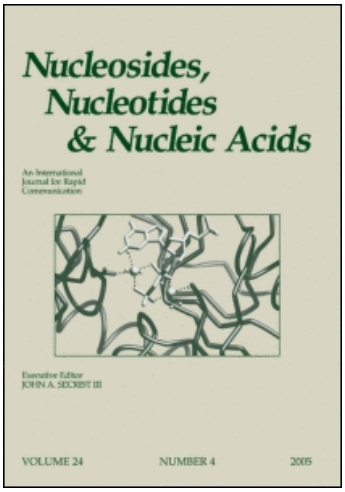

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SUBSTRATE/INHIBITOR PROPERTIES OF TUMOUR PURINE NUCLEOSIDE PHOSPHORYLASE.

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Abstract - Substrate/inhibitor properties of purine nucleoside phosphorylase (PNP), isolated from human lung and kidney tumour tissues, have been characterised and compared with those of the enzyme from the corresponding normal organs.

Malignant tissues are known to exhibit a variety of enzyme abnormalities. Purine nucleoside phosphorylase (PNP), (E.C.2.4.2.1.), a key enzyme in purine metabolism, has been reported, in conjunction with adenosine deaminase (ADA), (E.C.3.5.4.4.), to be a useful supplementary diagnostic marker for leukemia, and the ratio ADA/PNP permits discrimination between myeloid and monocytoid subtypes of acute myeloid leukemia (1). However the K_m of inosine for PNP from leukemic tissue does not appear to differ from that for the enzyme from normal cells; and the decrease in PNP activity in leukemic cells may be the consequence of a modification of the regulatory mechanism for enzyme synthesis in the pathway of purine metabolism in leukemic cells (2). By contrast, peripheral blood null lymphocytes from AIDS patients have been reported to exhibit elevated levels of PNP and ADA, implying that cellular immunodeficiency in this instance is more likely associated with an increase in an immature and/or activated subset of lymphocytes (3).

We have compared the substrate/inhibitor properties of PNP isolated from human lung and kidney tumour tissues relative to the enzyme from the corresponding normal organs. The tumour tissues were collected from a clear cell carcinoma of the kidney and from adenocarcinoma of the lung. The procedure employed for isolation and partial purification of the enzyme is depicted in Table 1 (in this case kidney tumour PNP).

Table 1
Partial purification of kidney tumour PNP.

Purification step	Total volume (ml)	Protein (mg)	Total activity (U)[a]	Specific activity (U/mg)	Purification (fold)	Recovery (%)
Crude extract[b]	90	1044	22.5	0.021	1	100
Ammonium sulphate fractionation[c]	38	296	19	0.064	3.0	84.4
CM Sephadex[d]	7	56	4.3	0.076	3.6	18.9
Affinity chromatography[e]	6	0.36	1.5	4.2 f	198	6.6

[a]Enzyme activity evaluated from the amount of guanine released following 6 min incubation at 37 C. [b]In 20 mM phosphate buffer pH=6 + 1 mM DTT. [c]Successive 40% and 80% saturation. [d]Removal of hemoglobin as elsewhere described (4). [e]Sepharose CL-6B activated with 6-hydroxy-9-p-aminobenzylpurine (5). [f]Specific activities /U/mg/ for the other enzymes were: lung tumour - 1.5, lung - 1.8, kidney - 3.9.

Substrate requirements.

The level of PNP activity in the tumour tissues did not significantly differ from that in normal tissues. The enzymes from human lung (L) and kidney (K), as well as those from the corresponding tumour tissues (LT and KT) catalyse the phosphorolysis, like PNP from human erythrocytes, of inosine (Ino), guanosine (Guo), and the synthetic fluorescent substrate - N(7)-methylguanosine (m^7Guo),as shown in Table 2.

With Ino as substrate the enzyme from all four sources exhibits the substrate activation characteristic for PNP from various sources. This is reflected in the non-linear double-reciprocal plots of 1/v vs. 1/S when the rate of phosphorolysis is measured over a wide range of substrates concentrations. For the enzymes from normal kidney and lung tissues, and as previously noted for human erythrocytes (9) such substrate activation is observed at Ino concentration exceeding 200 uM. However, as shown in Table 2., such activation for the two tumour enzymes manifests itself at inosine concentrations of about 40 uM. For the L, K, and KT enzymes, extrapolation of the linear portion of the plot for low concentrations of Ino, as for PNP from human erythrocytes (9) leads to a Michaelis constant Km ~30uM, whereas that for the enzyme from LT is 3-fold lower (K_m~11 uM). The synthetic substrate m^7Guo differs from the natural substrate Ino in that it shows no substrate activation for all four enzymes. But the K_m is lower for the enzyme from normal tissue (K_m = 7-8 uM) relative to that for the enzyme from the tumours (K_m = 14-15 uM). Furthermore, the V_{max} for the kidney tumour enzyme with m^7Guo as substrate is 40% higher than with Ino, whereas for the enzyme from normal kidney it is 60% lower.

Table 2

Kinetic constants for phosphorolysis of inosine and N(7)-methylguanosine by PNP purified from kidney and lung normal and tumour tissues. Phosphorolysis of Ino and m^7Guo was monitored in 50 mM phosphate buffer pH 7.0 at 25 C, spectrophotometrically. For m^7Guo the direct method, and for Ino, coupling with xanthine oxidase (6) was employed. Kinetic parameters were obtained from initial velocities (7) and by continous monitoring (7,8).

PNP	Substrate	Substrate concentration (uM)	K_m (uM)	V_{max} (%)[a]
Kidney normal (K)	Inosine	< 100	32	100
		< 200 [b]	41	148
	N(7)-Methylguanosine	[c]	7	41
Kidney tumour (KT)	Inosine	< 40	28	100
		> 40 [b]	115	~189
	N(7)-Methylguanosine	[c]	14	137
Lung mormal (L)	Inosine	< 100	34	100
		< 200 [b]	42	140
	N(7)-Methylguanosine	[c]	8	93
Lung tumour (LT)	Inosine	< 22	11	100
		> 40 [b]	94	254
	N(7)-Methylguanosine	[c]	15	62

[a]For each enzyme the 100% value for Ino was that obtained at a concentration below that for substrate activation. [b]Substrate activation above this concentration. [c]No detectable substrate activation.

Inhibition by acyclonucleosides.

Susceptibility to inhibition of all four enzymes was examined with the use of a series of 12 acyclonucleoside analogues with known inhibitory properties vs human erythrocyte PNP (10). The apparent inhibition constants, K_i^{app}, are listed in Table 3. which shows several instances of differences in affinity for the normal and tumour enzymes. For example compound 12 is a weaker inhibitor (4-5 fold) of the two tumour enzymes, and the kidney tumour enzyme is, relative to the normal kidney enzyme,

Table 3

Inhibition of PNP from normal and tumour kidney and lung tissues by some known acyclonucleoside inhibitors in 50 uM phosphate buffer pH 7.0 at 25 C. K_i values were determined with Ino as the variable substrate by continous spectrophotometric monitoring (7,8) and the coupled xanthine oxidase procedure (6). K_i^{app} values were derived from the increase of K_m^{app} and decrease of (V_{max}/K_m) for Ino phosphorolysis in the presence of the inhibitor.

Analogue	K_i^a (uM)	K_i^{app} (uM) Kidney		K_i^{app} (uM) Lung	
		tum.	norm.	tum.	norm.
1 9-(2-methyl-3,4-dihydroxybutyl)guanine	1.8	1.2	1.5	3.2	1.3
2 9-(2-metoxy-3,4-dihydroxybutyl)guanine	2.8	1.5	2.1	2.2	1.2
3 9-(2-fluoro-3,4-dihydroxybutyl)guanine	3.5	1.7	1.6	3.5	3.4
4 9-(2-fluoro-3,4-dihydroxybutyl)hypoxan thine	25	44	48	33	21
5 9-(4-hydroxybutyl)guanine	52	32	69	52	84
6 9-(4-hydroxybutyl)adenine	>100	>100	b	63	b
7 9-(3,4-dihydroxybutyl)guanine	21	4.2	6.7	9.3	5.2
8 7-methyl-9-(3,4-dihydroxybutyl)guanine	43	>200	300	210	110
9 8-bromo-9-(3,4-dihydroxybutyl)guanine	3.7	0.5	2.3	4.4	2.8
10 8-amino-9-(3,4-dihydroxybutyl)guanine	4.3	6.0	5.3	5.4	4.1
11 9-(3-carboxy-4-hydroxybutyl)guanine	>200	140	b	140	b
12 9-(3-metoxy-4-hydroxybutyl)guanine	~100	>400	75	230	60

[a]K_i for inhibition of human erythrocyte PNP (10), [b]Not determined.

inhibited 5-fold more effectively by compound 9. While the overall results point to the absence of significant differences in kinetic properties between the enzymes from normal and malignant tissues, the differences in substrate activation and response to inhibitors are of some interest. More extensive purification of the enzymes would be required to better define these defferences.

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