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QUARTERLY

Substrate specificity of methylthioadenosine phosphorylase from human liver*

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Methylthioadenosine (MTA) phosphorylase purified 615-fold from human liver cleaved phosphorolytically nucleoside analogues at the decreasing specific activity: 5'-deoxyadenosine > 5'-iodo-5'-deoxyadenosine > MTA > adenosine > 2-chloroadenosine > 2-chloro-5'-*O*-methyl-2'-deoxyadenosine > 2-chloro-2'-deoxyadenosine > > 2'-deoxyadenosine. Adenosine and analogues of 5'-deoxyadenosine were strong competitive inhibitors of MTA phosphorolysis catalysed by the human liver enzyme.

Methylthioadenosine (MTA)[1] phosphorylase (EC 2.4.2.28) catalyses the phosphorolytic cleavage of methylthioadenosine to adenine and methylthioribose-1-phosphate. MTA is a natural nucleoside formed from *S*-adenosylmethionine by at least five independent pathways [1]. Recently, it has been found that some cell lines derived from leukemias, lymphomas, some solid tumors or gliomas are deficient in MTA phosphorylase [2–4]. Additional information that in a number of leukemia cell lines, MTA phosphorylase deficiency accompanies the deletion of the interferon genes of chromosome 9 [5], have increased the interest in the role of this enzyme in the genetic mechanisms of neoplastic disorders. The question arises whether the perturbation in MTA phosphorylase activity disturbs *S*-adenosyl-methionine-dependent transmethylation reactions [6]. This seems to be particularly important in cells with a deficiency of MTA phosphorylase. It would be interesting to examine to what extent synthetic adenosine analogues blocking the MTA phosphorylase activity influence the recycling of adenine which is released not only from MTA but also from adenosine and probably from 2'-deoxyadenosine derived from DNA catabolism. In mammalian cells, phosphorolytic activity *vs* adenosine was shown to be very low [7–9]. In other species, e.g. parasitic worm *Schisostoma mansoni* [10], and hepatopancreas from the gastropod *Helix pomatia* [11], this activity is much higher. Our previous studies showed incorporation of the adenine moiety derived from dAdo into ATP in intact human erythrocytes [12, 13]. An interaction of dAdo with *S*-adenosylhomocysteine hydrolase (SAH) was suggested, however, the generation

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[1]Abbreviations used: Ado, adenosine; dAdo, 2'-deoxyadenosine; DTT, dithiothreitol; MTA, methylthioadenosine; SAH, *S*-adenosylhomocysteine.

of adenine from dAdo by the action of MTA phosphorylase could constitute the alternative explanation [12].

In the present study we have examined a number of compounds with special regard to their inhibitory and substrate properties. MTA phosphorylase used was only partially purified as a side product of SAH hydrolase isolated from human liver [14]. Therefore it was of lower purity than the enzyme isolated by Toorchen & Miller [8].

MATERIALS AND METHODS

Chemicals. [8-^{14}C]Ado, [8-^{14}C]dAdo, MTA, Ado, dAdo and most of adenosine analogues were products of Sigma Chemical Co. (Pade, U.K.). Neplanocin A was a kind gift from Dr T. Saito (Toyo Tozo Co. Ltd, Japan). 2-Chloro-2'-deoxyadenosine was obtained from Dr P. Grieb (Department of Neurophysiology, PAN, Warsaw, Poland), and ribavirin from ICN Pharmaceuticals (U.K.), Sephacryl S-300 and DEAE Sephacel were products of Pharmacia Fine Chemicals (U.K.).

Enzyme assays. MTA phosphorylase activity was assayed in the phosphorolytic direction as described previously [12, 13]. The standard incubation mixture (0.275 ml) contained (at final concentrations): 0.5 mM MTA, 30 mM phosphate buffer (pH 7.4), 1 mM DTT, the enzyme preparation (60–70 µg of protein per sample) and 1 mM the tested adenosine analogue used as inhibitor. The mixture was preincubated for 30 min at 37°C, the reaction was started by addition of MTA, and the incubation was continued for 30 min. The reaction was terminated by the addition of 25 µl of 40% trichloroacetic acid, the precipitated protein was removed by centrifugation and the supernatant extracted with water-saturated diethyl ether. Enzyme activity was determined as the amount of adenine released, using a Millipore Waters trimodulator HPLC system with a 0.45 cm × 12.5 cm, 5 µ ODS-2 column (Hichrom, Reading, England). Adenine was separated from substrate and inhibitors, using an isocratic phosphate buffer elution (KH_2PO_4, 1 g/l), pH 4.2, containing 20% methanol. In several experiments, 8-^{14}C-labelled nucleosides (final concentration 0.5 mM) were used to check the enzyme specificity *vs* Ado or dAdo, and two methods were used for analysis: HPLC system linked to in-line radiodetection, or descending paper chromatography (Whatman 1) in 2 mM ammonium formate (R_F for Ado, dAdo, AMP, dAMP and adenine of 0.57, 0.61, 0.84, 0.85, 0.40, respectively).

S-Adenosylhomocysteine hydrolase, adenosine deaminase and purine nucleoside phosphorylase were assayed as described previously [14, 15].

Enzyme preparation. MTA phosphorylase was partially purified from the cytosolic fraction of human liver in the course of simultaneous purification of SAH hydrolase [12]. The following modifications were used: after removal of the precipitate containing SAH hydrolase (0.35–0.50 ammonium sulfate saturation fraction), MTA phosphorylase was precipitated at 0.50–0.70 ammonium sulfate saturation. The same columns as for SAH hydrolase were used for the subsequent purification steps, however, the Sephacryl S-300 column was used prior to the DEAE Sephacel column. The enzyme from the last column was eluted by phosphate buffer (10 mM), pH 7.4, containing DTT (1 mM) with a linear gradient of KCl (up to 0.1 M).

All the kinetic parameters [16] and protein [17] were estimated as described previously [14].

RESULTS AND DISCUSSION

Characterization of partially purified human liver MTA phosphorylase

Purification of MTA phosphorylase was aimed not at preparation of homogeneous protein but at obtaining an enzyme free of any contamination by other enzymes which could metabolize adenosine or its analogues, namely SAH hydrolase, ADA or PNP.

The enzyme was purified approximately 615-fold and its relative molecular mass was estimated by Sephadex gel filtration to be 54000. This value was in agreement with M_r of the highly purified MTA phosphorylase from the same tissue [8], but was lower than that of the enzyme from human placenta (95000) [18].

The specific activity of the isolated human liver MTA phosphorylase in the direction of nucleoside cleavage was 5.5 µmoles/mg protein per hour [8, 18]. The apparent K_m value for

MTA estimated from six substrate concentrations using the Harvard-Graphics plotting program, obeying Hanes and Lineweaver-Burk kinetics, was 1.3×10^{-5} M. This value is comparable to K_m of human prostate enzyme (2.5×10^{-5} M) [19], but higher than the K_m value found previously [8] for the human liver enzyme (0.15×10^{-5} M). These differences might be explained by the presence of some factors which could be critical for the kinetic behavior of the enzyme *in vitro* (among others, the ratio of phosphate, DTT and substrate concentrations [8]).

Inhibitory effect and substrate specificity

The influence of the presence of adenine and other purine bases or adenosine and its analogues on the activity of isolated MTA phosphorylase toward MTA is presented in Table 1. Analogues without oxygen in 5′-position, i.e. 5′-deoxyadenosine and 5′-iodo-5′-deoxyadenosine (both at 1 mM conc.), proved to be very strong competitive inhibitors of MTA phosphorylase [20]; they acted also as substrates (Table 2), with K_m values lower than for MTA. It is worth mentioning that adenine and 5′-deoxyadenosine completely inhibited MTA phosphorylase activity (Table 1). The results concerning 5′-dAdo confirm the previous data, that 5′-dAdo and MTA are alternative, competing substrates for the same catalytic site of MTA phosphorylase [20]. The comparison of K_m values shows that the enzyme has relatively high affinity to 5′-deoxy analogues of adenosine. This fact indicates that a polar group in the 5′-position reduced the binding affinity of MTA phosphorylase, as reported previously [8, 9].

The examination of the inhibitory effect demonstrated that adenosine caused 92% inhibition of the isolated enzyme, while the specific activity towards adenosine was approximately 1/8 that towards the natural substrate, i.e. MTA, and K_m value for adenosine was one order of magnitude higher (Table 2). These results are comparable to reported data [8]. The activity of MTA phosphorylase towards the chloro-analogues is low, and the presence of either 2-chloroadenosine or 2-chloro-2′-deoxyadenosine in the reaction mixture did not affect the adenine release from natural substrate, MTA. The enzyme activity towards dAdo was found only when special conditions of incubation were applied (i.e. reaction time up to 2 h, and higher protein concentration) and it was approximately 90-fold smaller as compared to MTA. In turn, 2′,3′-dideoxyadenosine was not phosphorolysed by human liver MTA phosphorylase. The phosphorolytic cleavage of dAdo to adenine remains still obscure. Previously [14, 21], we have suggested that adenine release in erythrocyte lysates is a result of SAH hydrolase-dAdo complex cleavage. More recently the degradation of dAdo to adenine has been also observed in human and opossum erythrocyte lysates [22]. The same observations were reported for the isolated plant SAH hydrolase [23], and for another enzyme, adenosine phosphorylase isolated from hepatopancreas of the gastropod *Helix pomatia* [11]. The latter enzyme is inactive with MTA as substrate. It is improbable that the phosphorolytic cleavage of dAdo is catalysed by PNP, because the release of adenine was observed in the lysate of erythrocytes deficient in PNP [24]. Previously, we supposed that adenine could be the product of catalytic cleavage of adenosine by MTA phosphorylase. Although the present results seem to confirm this possibility it appears to be excluded by the documented release of adenine from dAdo in a mycoplasma-free human T-lymphoid cell line

Table 1

Human liver MTA phosphorylase activity in the presence of adenosine analogues.

The enzyme activity in the presence of the indicated 1 mM adenosine analogue added as inhibitor, as percentage of the activity with the substrate (0.5 mM MTA) alone. Results are the average of at least four experiments. The differences between separate experiments did not exceed 15%.

Inhibitor	% of control activity
No analogue (control) MTA	100
5′-Deoxyadenosine	0
5′-Iodo-5′-deoxyadenosine	13
Adenosine	8
2′-Deoxyadenosine	100
2′,3′-Dideoxyadenosine	100
2-Chloroadenosine	100
2-Chloro-2′-deoxyadenosine	100
Adenine	0
Guanine	82
Xanthine	100
Hypoxanthine	100

Table 2

Substrate specificity of MTA phosphorylase from human liver.

Each value represents the mean ± SEM for four experiments. The enzyme activity was determined as described in Materials and Methods.

Substrate	MTA phosphorylase activity (μmol/mg protein per h)	K_m (M)
MTA	5.5 ± 0.4	1.3×10^{-5}
5′-Deoxyadenosine	15.7 ± 1.1	4.3×10^{-6}
5′-Iodo-5′-deoxyadenosine	12.8 ± 0.9	5.6×10^{-6}
Adenosine	0.69 ± 0.05	6.6×10^{-4}
2′-Deoxyadenosine	0.06 ± 0.005	–
2′,3′-Dideoxyadenosine	not detected	–
2-Chloroadenosine	0.66 ± 0.05	6.8×10^{-4}
2-Chloro-2′-deoxyadenosine	0.60 ± 0.04	7.5×10^{-4}
2-Chloro-5′-*O*-methyl-2′-deoxyadenosine	0.63 ± 0.05	7.2×10^{-4}

CEM, which has no MTA phosphorylase activity [24].

The present results, demonstrating that partially purified MTA phosphorylase from human liver catalyses the phosphorolytic cleavage of both MTA analogues (5′-dAdo and 5′-iodo-5′-dAdo) and adenosine analogues (2-chloro-adenosine, 2-chloro-2′-deoxyadenosine), suggest that this enzyme could be responsible for the catabolism of at least some dAdo derivatives to adenine under appropiate conditions. The activity of MTA phosphorylase towards dAdo is, however, much lower than towards the natural substrate MTA. It seems that two interpretations of this result are possible: either the metabolism of dAdo to adenine depends on the type of tissue, or under special conditions (i.e. in the genetic absence of ADA or in the presence of 2′-deoxycoformycin and 5′-iodotubercidin, inhibitors ADA and Ado kinase, respectively) adenine may be released from dAdo moiety by MTA phosphorylase or by SAH hydrolase.

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