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Physicochemical and Immunological Studies on Mammalian 5'-Deoxy-5'-methylthioadenosine Phosphorylase*

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5'-Deoxy-5'-methylthioadenosine phosphorylase (MTAase) was purified to homogeneity (10,000-fold) from bovine liver with a recovery of 12%. The pure protein shows a molecular weight of about 98,000 ± 3,000 and is composed of three apparently identical subunits. Several physicochemical features have been investigated including hydrodynamic properties, amino acid composition, and secondary structure. In particular, the CD spectrum of the protein indicates a very low α-helical content and a large percent of β-structure and random coil.

The pure protein was used to raise specific rabbit antisera but, because of the scarce antigenic properties of the native enzyme, different chemically modified forms were prepared and employed as immunogens. Among the antibodies obtained, those to keyhole limpet hemocyanin-MTAase recognize both the native and the denatured enzyme and are also active against the human protein. Therefore, they were employed as a tool to investigate the occurrence of inactive forms of MTAase in two human malignant cell lines lacking this enzymatic activity. The results obtained with K562 and Jurkat cells indicate that the protein is absent in these phosphorylase-deficient cell lines.

The importance of a detailed characterization of mammalian 5'-deoxy-5'-methylthioadenosine phosphorylase (MTAase)[1] has greatly increased since the discovery of a possible relationship between deficiency of this enzyme and malignancy. Indeed, while MTAase has been found in all normal tissues and in cell lines of nonmalignant origin, a number of transformed cells lack this enzymatic activity (1–4). In particular, MTAase was not detectable in more than 20% of cell lines derived from various tumors including human leukemia, malignant melanoma, and breast cancer (2). Moreover, the deficiency has been demonstrated in 12 out of 120 cases of human acute leukemias studied, where it appeared as an inherent feature of the malignant blast cells and was not shared by normal cells (3, 4).

MTAase (5'-deoxy-5'-methylthioadenosine:orthophosphate methylthioribosyltransferase, EC 2.4.2.28) catalyzes the phosphorolytic cleavage of MTA, a sulfur adenosyl nucleoside formed from *S*-adenosylmethionine by several independent pathways (5, 6). The reaction products, adenine and 5-methylthioribose 1-phosphate, are recycled in turn to AMP and methionine, respectively (7, 8). It is worth mentioning, in this context, that the enzymatic breakdown of MTA represents the main reaction leading to endogenous adenine (9), thus suggesting a critical role for MTAase in a purine salvage pathway.

The molecular mechanism(s) responsible for the absence of MTAase activity only in malignant cells is still obscure. Indeed, the phosphorylase deficiency, as assessed by enzymatic assays and autoradiography, could be due either to the lack of expression of the gene coding for the protein or to the synthesis of nonfunctional enzyme. An alteration on the short arm of chromosome 9, where both a known fragile site and the putative gene of the enzyme are localized (10), was found in one case of lymphomatous acute lymphoblastic leukemia associated with MTAase deficiency (11). On the basis of this observation, Carson *et al.* (12) hypothesized that chromosomal deletion or translocation might be responsible for both the absence of enzyme activity and the loss of a tumor suppressor gene. Conversely, a recent study carried out on a large number of acute leukemias failed to detect evident aberrations at chromosome 9 in more than 80% of patients with the enzyme deficiency (4). However, the lack of identifiable karyotypic changes is not sufficient to exclude minor chromosomal alterations. Only the availability of molecular probes, namely specific cDNA and antibodies anti-MTAase, will permit the precise identification of the mechanisms underlying the enzyme deficiency.

Although many reports on this enzyme have been published (13–15), the mammalian phosphorylase has been purified to homogeneity and partially characterized only from human placenta (16). However, a more detailed investigation on the human enzyme has been hampered by the scarce amount of MTAase recovered after the 30,000-fold purification required to obtain the pure protein.

This paper describes the purification to homogeneity and reports several physicochemical properties of MTAase from bovine liver. This tissue, indeed, is a more suitable source for preparing substantial amounts of enzyme. Furthermore, high titer specific antisera were raised in rabbits employing as immunogens different forms of chemically modified bovine enzyme. The antibodies recognized multiple determinants on the protein and reacted with both bovine and human MTAase, thus allowing investigation on the absence of MTAase activity in two human malignant cell lines.

EXPERIMENTAL PROCEDURES

Chemicals—*S*-Adenosylmethionine was prepared from *Saccharomyces cerevisiae* and isolated by ion exchange chromatography (17).

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[1] The abbreviations used are: MTAase, 5'-deoxy-5'-methylthioadenosine phosphorylase; MTA, 5'-deoxy-5'-methylthioadenosine; KLH, keyhole limpet hemocyanin; SDS, sodium dodecyl sulfate; HPLC, high pressure liquid chromatography.

S-Adenosyl-L-[*methyl*-^{14}C]methionine was supplied by Radiochemical Centre, Amersham, Bucks, United Kingdom. MTA and [*methyl*-^{14}C]MTA were prepared from labeled and unlabeled *S*-adenosylmethionine (18) and purified by HPLC (19). Keyhole limpet hemocyanin (KLH), protein A-agarose, complete and incomplete Freund's adjuvant, and cyanogen-activated agarose were supplied by Sigma. DEAE-Sephacel, Sephacryl S-200, PBE 94, and Polybuffer 74 were from Pharmacia-LKB, Uppsala, Sweden. Protein A-horseradish peroxidase conjugate and 4-chloro-1-naphthol were obtained from Bio-Rad. All other chemicals were of the purest commercial grade.

Enzymatic Assays—The activity of MTAase was determined by measuring the formation of [*methyl*-^{14}C]5-methylthioribose-1-P from [*methyl*-^{14}C]MTA (16).

Purine nucleoside phosphorylase activity was assayed following the formation of hypoxanthine from inosine. The standard reaction mixture contained (in a total volume of 0.5 ml) 50 mM potassium phosphate buffer, pH 7.4, 1 mM dithiothreitol, 1 mM inosine, and the enzymatic extract. After incubation, the reaction was stopped by heating at 100 °C and centrifuged. An aliquot of supernatant was analyzed by the reversed-phase HPLC column employing as eluent 2.5 mM potassium phosphate buffer, pH 3.0, 2.5% methanol at a flow rate of 1.5 ml/min. Hypoxanthine and inosine were eluted after 2.6 and 8.3 min, respectively.

One unit of the enzyme is defined as the amount of enzyme which catalyzes the phosphorolytic cleavage of 1 μmol of the substrate at 37 °C in 1 min.

Purification Procedure—Unless otherwise stated, all procedures were carried out at 4 °C. 1 kg of bovine liver was homogenized in a Waring Blendor with 4 volumes of 10 mM potassium phosphate buffer, pH 7.4, containing 1 mM dithiothreitol (buffer A) and centrifuged for 1 h at 20,000 × *g*. The postmitochondrial sample was brought to pH 5.2 by dropwise addition of 1 M acetic acid and centrifuged for 15 min at 20,000 × *g*. After adjustment to pH 7.4, the supernatant was heated at 56 °C for 5 min and the copious precipitate removed by centrifugation at 15,000 × *g* per 30 min. The sample was then subjected to ammonium sulfate fractionation, acetone precipitation, and DEAE-Sephacel chromatography as described for the placental enzyme (16). The ion exchange fractions containing at least 0.15 units/mg protein were pooled, concentrated by ultrafiltration, and dialyzed extensively against 25 mM imidazole HCl, pH 7.4, and 1 mM dithiothreitol. The sample was then loaded on a chromatofocusing column (PBE 94) (0.7 × 30 cm) equilibrated in the same buffer. The elution was carried out at a flow rate of 6 ml/h with Polybuffer 74, pH 4.0, diluted 1:10 in H_2O. MTAase activity was recovered at pH 5.6 which corresponds approximately to its isoelectric point. The fractions with a specific activity higher than 1.8 units/mg protein were pooled, adjusted to pH 7.4, and concentrated by ultrafiltration. Final purification was achieved by Sephacryl S-200 (1.5 × 96 cm) equilibrated with 50 mM potassium phosphate buffer, pH 7.4, 1 mM dithiothreitol, and 0.2 M NaCl. Elution was carried out at a flow rate of 2 ml/h with the same buffer. The fractions with a specific activity higher than 8 units/mg protein were analyzed by SDS-gel electrophoresis and those showing a single band were pooled and concentrated by ultrafiltration. The sample was then dialyzed against buffer A containing 20% glycerol and stored at −20 °C.

Polyacrylamide Gel Electrophoresis—SDS-polyacrylamide gel electrophoresis was carried out at room temperature by using either 12.5 or 15% acrylamide resolving gel and a 5% acrylamide stacking gel (20). After the electrophoretic separation, gels were either stained in 0.1% Coomassie Blue or used for immunoblotting.

Ultracentrifugal Measurements—The molecular weight of the protein was determined by equilibrium sedimentation employing MTAase samples at 0.2 mg/ml concentration (21). The equilibrium was checked by comparing the sedimentation profile at 6-h intervals. Before the calculations, the profiles were corrected for the base-line absorbance by accelerating the rotor to 40,000 rpm for 6 h and then decelerating to initial speed just before the scan.

MTAase samples were also used for determination of the sedimentation rate at a rotor speed of 40,000 rpm and a temperature of 20 °C. The data were analyzed as in Ref. 21.

Circular Dichroism—The circular dichroism spectra were recorded on a dichrometer at 20 °C with approximately 0.2 mg/ml bovine enzyme. The CD data were interpreted by the method of Greenfield and Fasman (22).

Amino Acid Analysis—The amino acid composition of pure bovine MTAase was estimated by single column analysis on an amino acid analyzer after hydrolysis for 24, 48, and 72 h in 6 M HCl at 110 °C. The concentrations of threonine, serine, valine, and tyrosine are extrapolated values. The cysteine concentration was determined by sulfydryl group titration employing dithionitrobenzoic acid (23). Tryptophan was determined by means of a second derivative UV spectrum of the unfolded enzyme (24).

Preparation of MTAase Antisera—Preliminary experiments showed that multiple subcutaneous injections of native bovine liver MTAase into rabbits resulted in the production of antisera with low titer. Therefore, in order to enhance the immunogenicity of the protein, different MTAase derivatives were prepared. Polymeric MTAase and MTAase linked to KLH were obtained by using glutaraldehyde as coupling agent (25). The analysis by SDS-polyacrylamide gel electrophoresis of the reaction mixture confirmed the conjugates formation. Moreover, about 0.5 mg of MTAase were coupled to 1.5 ml of swollen CNBr-activated agarose according to the manufacturer's instructions.

Polymeric MTAase, hemocyanin-MTAase, and agarose-MTAase were injected subcutaneously in New Zealand female rabbits in complete Freund's adjuvant. The amounts employed corresponded to 0.1 mg of MTAase for each of the antigens. Three subsequent injections were given at intervals of 4 weeks, and the rabbits were bled 7 days after the last injection. The IgG fraction of antisera was purified as follows. 1 ml of sample was extensively dialyzed against 50 mM potassium phosphate buffer, pH 7.4, and passed through a 2-ml protein A-agarose column equilibrated with the same buffer. The column was washed with the equilibration buffer plus 1.5 M NaCl, and the antibodies were eluted at pH 2.8 with 0.1 M glycine HCl. The IgG-containing fractions were adjusted to pH 7.4, pooled, concentrated, and stored at 4 °C in 0.1% NaN_3. Preimmune sera were taken before the primary injection.

Immunoprecipitation—Immunoprecipitation assays were carried out by overnight incubation at 4 °C of increasing amounts of antibodies and a fixed amount of antigen in 100 mM potassium phosphate buffer, pH 7.4, 1% bovine serum albumin, 1 mM dithiothreitol in a final volume of 100 μl. In order to assure the precipitation of the antigen-antibody complex, 20–40 μl of protein A-agarose (1:1 dilution in water) were added, and the mixture was further incubated for 2 h at 4 °C. The sample was then centrifuged at 15,000 × *g* for 5 min. The remaining enzymatic activity was measured in aliquots of the supernatant.

A similar procedure was used to investigate by Western blotting the immunoreactive material occurring in the liver homogenate. 70-mg proteins of 20,000 × *g* supernatant were incubated for 4 h at room temperature with 1.5 mg of anti-KLH-MTAase IgG in a total volume of 1.2 ml. The immunocomplexes were precipitated by incubation with 400 μl of protein A-agarose for 1 h at room temperature. After centrifugation, the gel was washed 3-fold with 1 ml of 20 mM Tris-HCl, 0.5 M NaCl, pH 7.4, and the proteins were eluted with 2 ml of 0.1 M glycine HCl, pH 2.8. The sample was adjusted to pH 7.4, lyophilized, and then analyzed by SDS-polyacrylamide gel electrophoresis and immunoblotting.

Immunoblotting—After separation on SDS-polyacrylamide gel electrophoresis the proteins were electrophoretically transferred to a nitrocellulose sheet at a constant current of 200 mA in a Bio-Rad Trans-Blot cell for 4 h. The transfer was performed in 20 mM Tris-HCl, 0.19 M glycine, 20% methanol, pH 8.3. After the blocking of the unbound sites with gelatin, the filter was incubated for 2 h with the antiserum against MTAase diluted 1:1000 with Tris-buffered saline containing 1% gelatin. Finally, the immunocomplexes were revealed by employing horseradish peroxidase-protein A following the manufacturer's instructions.

RESULTS

Purification Procedure and Physicochemical Properties—The procedure for purification of bovine liver MTAase employs several preliminary batch steps, taking advantage of the remarkable stability of the enzyme against acid and thermal denaturation (Table I). Moreover, the chromatofocusing represents the major modification of the procedure previously developed for the placental enzyme. Approximately 2 mg of purified enzyme were obtained per kg of bovine liver in about 12% yield. The final specific activity (four different preparations) ranged between 9 and 12 μmol of MTA cleaved per min per mg of protein. This represents more than a 10,000-fold purification over the crude supernatant. The protein was stable for up to 6 months after storage at −20 °C in 50 mM

TABLE I

Purification of MTAase from bovine liver

	Total protein	Specific activity	Yield	Purification
	mg	*units/mg*[a]	%	*-fold*
Supernatant at 20,000 × *g*	203,000	0.0010	100	1
pH 5.2 treatment	119,000	0.0017	99.5	1.7
Heat treatment	66,000	0.0027	88.6	2.7
Ammonium sulfate (45-70%)	36,000	0.0034	66.0	3.4
Acetone (40-60%)	20,600	0.0060	61.0	6.0
DEAE-Sephacel	696	0.17	58.1	170
Chromatofocusing	21	2.1	21.6	2,100
Sephacryl S-200	2.4	10.3	12.3	10,300

[a] One unit is the amount of enzyme which cleaves 1 μmol of MTA/min at 37 °C.

TABLE II

Physicochemical properties of bovine liver MTAase

Molecular weight	
By gel filtration	96,000 ± 3,000
By sedimentation equilibrium	98,000 ± 3,000
Subunit molecular weight	32,000 ± 1,000
Sedimentation coefficient, $s_{20,w}$ ($\times 10^{-13}$ cm/s)	6.11
Stokes radius (Å)	1.22
Frictional ratio	4.5
Axial ratio	1:4
Secondary structure	
% α-helix	5
% β-structure	48
% random coil	47

potassium phosphate, pH 7.4, 1 mM dithiothreitol, 20% glycerol.

The homogeneity of the purified protein was evaluated by polyacrylamide gel electrophoresis under native and denaturing conditions as well as by isoelectrofocusing. By both approaches a single band was observed (data not reported). Moreover, the presence of only one symmetrical boundary in the sedimentation velocity experiments confirmed the purity of the protein.

Table II summarizes the main physicochemical characteristics of the bovine liver MTAase. The molecular weight of the native protein calculated by analytical ultracentrifugation is 98,000 ± 3,000. A similar value has been estimated by gel filtration on Sephacryl S-200 using both the pure enzyme and the 20,000 × *g* supernatant, thus suggesting that no artifact occurred during the purification procedure.

The electrophoretic pattern of bovine liver enzyme under denaturing conditions showed no evidence of subunit heterogeneity. From plots of migration of marker protein *versus* their molecular weight, the average M_r of MTAase subunits was estimated to be 32,000 ± 500.

By means of circular dichroism the secondary structure of the protein was investigated; the α-helix and β-structure contents were estimated to be approximately 5 and 48%, respectively, with about 47% random coil.

Finally, the amino acid composition of pure bovine liver MTAase is reported in Table III.

Immunological Properties—Initial attempts to obtain high titer antisera to native MTAase failed although several animals were employed, namely rabbits, guinea pigs, and goats. Therefore, two different strategies, mainly based on coupling of the protein with other macromolecules, were used to enhance the immunogenicity of MTAase. The first procedure, which employes glutaraldehyde as the coupling agent, allowed the preparation of either soluble polymers of MTAase or

TABLE III

Amino acid composition of MTAase from bovine liver

Amino acid	Residues/mol	Residue weight
		%
Asp	75	8.82
Thr	51	5.27
Ser	84	7.55
Glu	93	12.27
Pro	45	4.47
Gly	75	4.37
Ala	81	5.88
Val	57	5.78
Met	15	2.01
Ile	57	6.59
Leu	72	8.33
Tyr	21	3.50
Phe	27	4.04
His	24	3.36
Lys	60	7.86
Arg	36	5.75
Trp	15	2.85
Cys	12	1.27

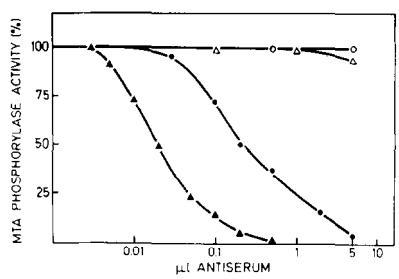

FIG. 1. **Immunoprecipitation assay of bovine liver MTAase.** Purified MTAase (5 ng) were incubated with increasing amounts of different antisera. After incubation with protein A-agarose and centrifugation, aliquots of supernatant were assayed for the phosphorylase activity. Antisera to: KLH-MTAase (▲——▲); agarose-MTAase (●——●); polymeric MTAase (△——△); control serum (○——○).

KLH-MTAase conjugates. Alternatively, the protein was linked to cyanogen-activated agarose. The three different antigens were then injected into rabbits following standard procedures.

Fig. 1 shows the immunoprecipitating effect of three of the antisera obtained. Antisera against KLH-MTAase and agarose-MTAase both recognize the native enzyme, even if at different titers, determining the precipitation of all the phosphorylase activity. On the contrary, polymeric MTAase antiserum, as well as preimmune serum, did not react at all with the native enzyme. When the antisera were evaluated by the dot blotting technique, antibodies directed against agarose-MTAase did not recognize the denatured enzyme, whereas the other two antisera gave a positive signal (data not reported). Therefore, only KLH-MTAase antiserum recognizes both the native and the altered form of the protein.

In order to characterize some of the interactions between the enzyme and the antibodies, the precipitating ability and the possible inhibitory effect on the phosphorylase activity of two different antisera, both produced against KLH-MTAase, were investigated. One of the two antisera showed both these effects, thus indicating that the binding with the antibodies resulted in a modification of the enzyme structure with a concomitant loss of catalytic activity (Fig. 2*A*). Conversely, a different behavior has been demonstrated for the other antiserum (Fig. 2*B*). Indeed, while precipitating capacity was significantly high, no inhibitory effect was detected. This indicates that the two antisera recognized, at least partially, different epitopes on the enzyme molecules. Because of its

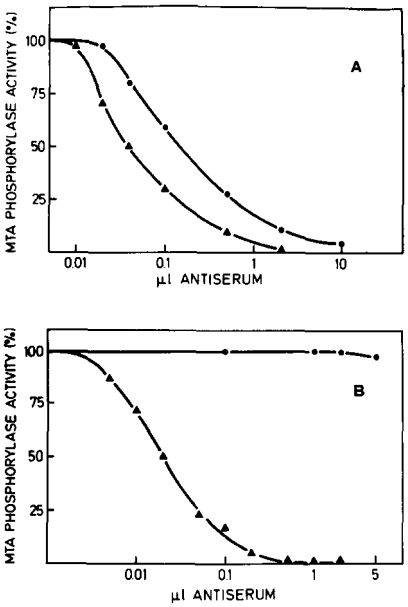

FIG. 2. **Inhibition and immunoprecipitation effects of two different anti-KLH-MTAase antisera.** Purified bovine liver MTAase (5 ng) was incubated with increasing amounts of two different rabbit antisera raised to KLH-MTAase. The activity was then determined either on aliquots of the samples (●——●) or on the supernatant after immunoprecipitation with protein A-agarose (▲——▲).

FIG. 3. **Immunoblots of MTAase from bovine liver.** *Lanes 1* and *3*, pure enzyme (200 ng); *lane 2*, 20,000 × *g* supernatant of bovine liver homogenate immunoprecipitated as described under "Experimental Procedures." *Lanes 1* and *2* were revealed with an anti-KLH-MTA phosphorylase antiserum and *lane 3* with a control serum. The *bars* on the *right* indicate molecular mass standards in kDa.

higher titer, the antiserum in Fig. 2*B* was employed in all the subsequent studies.

Immunoblotting of the homogeneous enzyme with the antibodies (Fig. 3, *lane 1*) revealed the presence of a single immunoreactive band. Due to the scarce amount of the protein, the immunoblotting analysis of the crude homogenate resulted in a 32-kDa faint band (data not shown). Therefore, the 20,000 × *g* supernatant was treated with a large excess of purified antibodies, and the immunoprecipitate was analyzed by immunoblotting. As shown in Fig. 3 (*lane 2*), only a strong signal at 32 kDa appeared with no other band detectable.

Because of the catalytic and structural similarity between mammalian MTAase and purine nucleoside phosphorylase (16, 26), cross-reactivity between anti-MTAase antibodies and purine nucleoside phosphorylase could be hypothesized. An immunoprecipitation assay, carried out employing 20,000 × *g* supernatant of bovine liver as the source of both the phosphorylases, excluded such a possibility (Fig. 4).

The antibodies to bovine liver MTAase were also able to precipitate the human enzyme, even if with a lower efficacy (Fig. 5). Moreover, immunoblotting analysis of a partially purified preparation of human enzyme gave a single band at about 32 kDa (data not shown). No precipitation was observed when *Escherichia coli* *S*-adenosylhomocysteine/MTA nucleosidase or *S. cerevisiae* MTAase was employed as the antigen (Fig. 5).

The observed immunoreactivity between antibodies to bovine MTAase and human enzyme gave us the possibility to investigate the occurrence of inactive forms of the phosphorylase in two MTAase-deficient cell lines, namely K562 (2) and Jurkat (27). Immunoprecipitation assays, carried out with

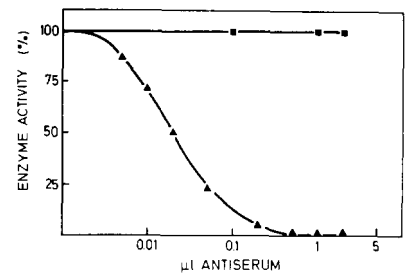

FIG. 4. **Immunoprecipitation of MTAase and purine nucleoside phosphorylase from bovine liver homogenate.** Approximately equal amounts of purine nucleoside phosphorylase (4 ng) and MTAase (5 ng), calculated on the basis of specific activity of the pure enzymes (present paper and Ref. 26), were incubated with increasing amounts of anti-KLH-MTAase antiserum. After incubation with protein A-agarose and centrifugation, aliquots of supernatants were assayed for MTAase (▲——▲) and purine nucleoside phosphorylase (■——■) activity.

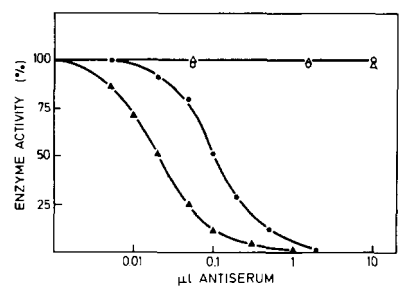

FIG. 5. **Immunoprecipitation assay of MTA-cleaving enzymes from various sources.** Equal amounts of enzymatic activity were immunoprecipitated as described under "Experimental Procedures." ○——○, *E. coli* *S*-adenosylhomocysteine/MTA nucleosidase; △——△, *S. cerevisiae* MTAase; ●——●, human MTAase; ▲——▲, bovine MTAase.

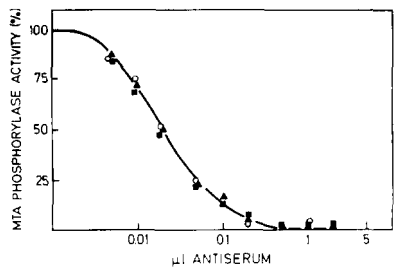

FIG. 6. **Immunoprecipitation assays of human MTAase in the presence and absence of K562 and Jurkat extracts.** Pure MTAase from human placenta (0.3 ng) was immunoprecipitated as described under "Experimental Procedures" in the absence (▲——▲) and in the presence of 500 μg of protein of K562 (○——○) and Jurkat (■——■) extract.

the human placental enzyme with and without an excess of MTAase-negative cell extract, gave superimposable results, hinting at the absence of cross-reacting material (Fig. 6). Identical results were obtained also with the other anti-KLH-MTAase serum and with the anti-agarose-MTAase serum (data not reported). Conversely, the addition of as low as 0.5 ng of pure human protein significantly modifies the immunoprecipitation curve. Moreover, control experiments, performed employing different amounts of HeLa extracts as phosphorylase-containing samples, gave a linear correlation between the enzyme content and the amount of the antiserum needed to achieve 50% loss of activity.

DISCUSSION

The understanding of the biochemistry and the molecular biology of MTAase has been gathering increasing interest since the discovery of a possible linkage between phosphorylase deficiency and malignant transformation. Previous attempts to investigate the molecular properties of the human enzyme were hampered by the scarce yield of purified protein obtainable from the human placenta (16). This paper describes a method to prepare substantial amounts of pure MTAase from bovine liver (Table I) and reports several physicochemical properties of the protein (Table II).

Like the human enzyme, bovine MTAase is a trimer of about 98 kDa composed of apparently identical subunits. This proteic structure, which is quite rare among the enzymes, is shared by mammalian purine nucleoside phosphorylase. The resemblance between the two phosphorylases can also be observed regarding other hydrodynamic features such as frictional ratio, axial ratio, and Stokes radius, as well as the secondary structure. Indeed a very low amount of α-helix and a relatively high percentage of random coil have been determined (Table II and Ref. 26). However, the lack of cross-reactivity between purine nucleoside phosphorylase and anti-MTAase immunoglobulins, as assessed by immunoprecipitation experiments, ruled out the occurrence of common epitopes and consequently of notable sequence homologies.

As already mentioned, a possible structural relationship between the gene of MTAase and a not yet identified tumor suppressor gene has been hypothesized (12). Thus the knowledge of the molecular mechanisms underlying the absence of enzyme activity might give some insight into the process of malignant transformation. In this context, the availability of high titer specific antisera represents an important tool. Previous attempts to obtain polyclonal anti-MTAase antibodies failed even if different animals were employed. The scarce immunogenicity of the native protein is probably due to a high degree of conservation among mammals. On the other hand, MTAase linked to KLH represents an efficacious immunogen, which allows the preparation of highly specific rabbit polyclonal antibodies interacting with both native and denatured enzyme. The only protein of liver homogenate recognized by the antiserum was indeed MTAase, as demonstrated by Western blotting analysis and immunoprecipitation experiments.

The antibodies to bovine enzyme did not precipitate *E. coli* *S*-adenosylhomocysteine/MTA nucleosidase and *S. cerevisiae* MTAase suggesting a significantly different antigenic structure for such MTA-metabolizing proteins. On the other hand, the remarkable cross-reactivity between the immunoglobulins to bovine enzyme and the human phosphorylase indicates a relevant similarity between the two proteins and allows the use of the antisera in investigating the expression of MTAase gene in human enzyme-deficient cells.

When purified immunoglobulins were employed to verify the occurrence of cross-reacting material in two deficient cell lines, no inactive forms of the enzyme were detected. This result could be due either to the lack of MTAase synthesis in enzyme-deficient cells or to the occurrence of modified form(s) with a low affinity toward the antibodies. Although no conclusive proof in favor of either of these hypotheses can be presented yet, the lack of reactivity with three different anti-MTAase antisera strongly supports the absence of inactive forms.

At the present, the precise molecular mechanism causing the absence of MTAase protein is not known. Only the availability of specific cDNA might give a conclusive answer. In this light, the methods reported for obtaining substantial amounts of pure protein (for peptide sequencing and oligonucleotide synthesis) and specific antibodies represent essential steps for a cDNA library screening. Future studies will be carried out to obtain such a nucleic acid probe and to investigate the genetic alteration responsible for MTAase deficiency and possibly for malignant transformation.

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