nature of the agonists used to stimulate $Ca^{2+}$ inflow and to substantial differences in the incubation conditions employed for the treatment of cells with EEDQ.

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# Purification of methylglyoxal bis(guanylhydrazone)-induced spermidine *N*-acetyltransferase from baby hamster kidney cells (BHK-21/C13)

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Acetylation is the rate-limiting step in the degradation of spermine to spermidine and spermidine to putrescine [1]. These naturally occurring polyamines are essential for cell growth, and it is thought that acetylation is a means whereby the cell can decrease its intracellular polyamine content under conditions of growth inhibition [2, 3]. Distinct acetyltransferase enzymes, characterized by other substrate specificities, are present in the cell cytosol and nucleus [4, 5]. Two nuclear *N*-acetyltransferase enzymes have been purified from calf liver. These enzymes acetylated both spermidine and spermine [6]. A carbon tetrachloride-induced cytosolic acetyltransferase enzyme has also been purified from rat liver. This enzyme is distinct from the nuclear one and also acetylates spermidine and spermine [7].

Methylglyoxal bis(guanylhydrazone) (MGBG), an inhibitor of polyamine biosynthesis, has been shown to stimulate spermidine and spermine acetyltransferase activity in rat liver and kidney [4] and in baby hamster kidney (BHK-21/C13) cells [8]. The drug was used as an anti-leukaemic agent in the 1960s, but its use was discontinued due to toxic side-effects [9]. Recently, however, MGBG has been re-

evaluated as an anti-cancer drug in combination with another polyamine biosynthesis inhibitor, α-difluoromethylornithine [10, 11]. The combination with other drugs allows for lower, less toxic doses of MGBG to be used. In this study we have purified the MGBG-induced cytosolic spermidine acetyltransferase from cultured BHK-21/C13 cells.

*Materials*

Horse serum and tissue-culture plastics were purchased from Gibco-BRL, (Paisley, U.K.). Dulbecco's modification of Eagle's medium, penicillin and streptomycin were from Flow Laboratories (Rickmansworth, U.K.). [$^3$H]Acetyl-CoA (sp. radioact. 5 Ci/mmol) and [$^{14}$C]MGBG (12 mCi/mmol) were from Amersham International (Amersham, U.K.). MGBG was from the Aldrich Chemical Co. (Gillingham, U.K.). Aprotinin (Trasylol) was from Bayer Ltd (Newbury, U.K.). Silver stain kit was from Bio-Rad Laboratories (Walford, U.K.). Sym-norspermidine (3,3$^1$-Diaminodipropylamine) was from Koch Light Ltd (Haverhill, U.K.). Diethyl aminoethyl cellulose was from Whatman Ltd (Maidstone, U.K.). All other biochemicals were from the Sigma Chemicals Co. (Poole, U.K.).

*Methods and Results*

The purification of the spermidine *N*-acetyltransferase (SAT) is a modification of the procedure described by Della Ragione and Pegg for the rat liver enzyme [7].

At each purification step spermidine acetyltransferase activity was determined by a modification [8] of the method of Matsui and Pegg [12]. Acetyltransferase assay products were analysed as described previously [8]. Protein was determined by the method of Lowry *et al.* [13].

BHK-21/C13 cells were grown routinely at 37° in monolayer culture in an atmosphere of $CO_2$/air (1:19) in Dulbecco's medium supplemented with 10% (v/v) horse serum. The cells were grown for 24 hr then exposed to MGBG (10 μM) for 72 hr. Stimulation of cytosolic spermidine acetyltransferase activity after 72 hr exposure to drug was approximately 6-fold. Cells were harvested using trypsin/versene (1:3, v/v) and sedimented at 800 $g_{av}$ for 3 min. The cells were homogenized in 2 volumes of 0.25 M sucrose, 50 mM Tris–HCl, pH 7.5, 25 mM KCl and 5 mM $MgCl_2$. Aprotinin (50 kI.U./mL) was added at this stage and was present in all subsequent buffers. The homogenate was centrifuged at 25,000 $g_{av}$ for 30 min and the supernatant removed and centrifuged at 100,000 $g_{av}$ for 1 hr. This supernatant was dialysed against 100 volumes of 50 mM Tris–HCl, pH 7.5 for 4 hr.

The dialysed sample was applied to a column (5 × 10 cm) of DEAE-cellulose (Whatman DE-52) previously equilibrated with 50 mM Tris–HCl, pH 7.5. The dialysed sample was applied at a flow-rate of 60 mL/hr and the column was washed with 150 ml of 50 mM Tris–HCl, pH 7.5, and eluted with a linear gradient of from 0.1 to 0.6 M NaCl at a flow rate of 80 mL/hr. Spermidine acetyltransferase activity eluted between 0.19 and 0.24 M NaCl.

The enzyme solution was applied to the sym-norspermidine-Sepharose (1 × 13 cm) column [7] previously equilibrated with 50 mM Tris–HCl, pH 7.5, at a flow rate of 40 mL/hr. The column was washed with 0.5 M NaCl in 50 mM Tris–HCl, pH 7.5 (800 mL). The eluting buffer was then changed to 0.8 M NaCl and 50 mM Tris–HCl, pH 7.5 (800 mL). Spermidine acetyltransferase activity was eluted with 5 mM spermidine in 0.8 M NaCl and 50 mM Tris–HCl, pH 7.5 (Fig. 1). Fractions 60–80 were concentrated by ultrafiltration and washed twice with 50 mM Tris–HCl, pH 7.5, containing 10 mM spermidine.

The enzyme preparation from MGBG-treated BHK cells was purified over 2000-fold with a yield of over 90% (Table 1). The enzyme solution gave a single band on SDS–PAGE [14] with subsequent silver staining [15]. Comparing its molecular weight with other known compounds [ovotransferrin (76–78,000); albumin (66,250); ovalbumin (45,000); carbonic anhydrase (29,500); myoglobulin (17,200); cytochrome *c* (12,300; BDH, Poole, U.K.)] the single band corresponded to a molecular weight of about 60,000. The purified enzyme acetylated both spermine and spermidine, with the former being the better substrate (result not shown). The enzyme was specific for polyamines and no activity was detected against other substrates (MGBG, hydralazine, sulphadimidine, isoniazid, *p*-aminobenzoic acid).

The preparation exhibited Michaelis–Menten kinetics (Fig. 2) with a $K_m$ of 640 μM for spermidine. MGBG decreased the $V_{max}$ of the preparation indicating non-competitive inhibition by MGBG with respect to spermidine.

The enzyme was stable for only about 48 hr at −80° in 50 mM Tris–HCl, pH 7.5, and 10 mM spermidine with a subsequent 50% loss of activity over 7 days.

The purified enzyme did not cross react with antiserum prepared against the rat liver enzyme [4] (results not shown).

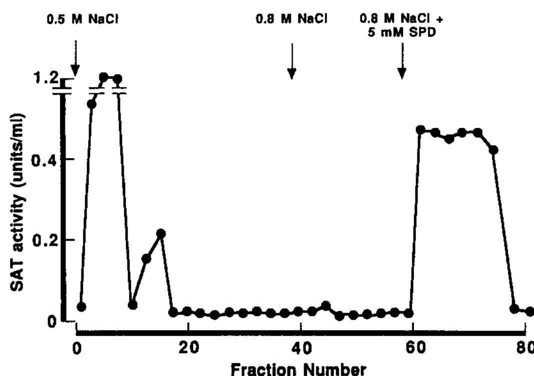


Fig. 1. Purification of MGBG-induced SAT by chromatography on sym-norspermidine Sepharose. The extract was applied to the column in 50 mM Tris–HCl, pH 7.5, and the column washed successively with 0.5 M NaCl, 0.8 M NaCl and 0.8 M NaCl plus 5 mM spermidine. Fractions (20 mL) were collected up to fraction 60 thereafter 8.7 mL fractions were collected. SAT activity was determined as described in the text.

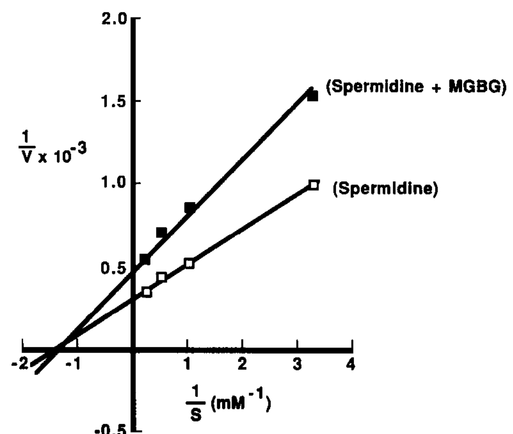


Fig. 2. Determination of $K_m$ for spermidine and the effect of MGBG on the purified enzyme. SAT activity was measured as described in the experimental section. MGBG was added at a final concentration of 1 mM.

Table 1. Purification of MGBG-induced spermidine/spermine N-acetyltransferase from BHK cells

| Purification step | Total activity (units) | Protein (mg) | Specific activity (units/mg protein) | Purification (× fold) |
|---|---|---|---|---|
| Homogenate | 0.51 | 190.8 | 2.67 | 1 |
| 25,000 supernatant | 0.46 | 26.1 | 17.8 | 7 |
| 100,000 supernatant | 0.39 | 0.52 | 758.9 | 284 |
| Dialysate | 0.16 | 0.24 | 682.3 | 255 |
| DEAE 52 cellulose | 2.40 | ND | — | — |
| Affinity chromatography | 3.01 | ND | — | — |
| Ultrafiltrate | 0.46 | 0.08 | 5791.7 | 2170 |

The purification steps are described in the text.
ND, not detected (samples were too dilute).
One unit is defined as 1 nmole of acetylpolyamine formed per min.

*Discussion*

The specific activity of this preparation was lower than that reported for the rat liver enzyme [7] but was higher than that reported by Libby [6] for the calf liver. No loss of $N^1$-acetylspermidine was observed when it was incubated with the enzyme preparation indicating there was no copurification of a deacetylase activity.

MGBG itself did not appear to be a substrate for the purified enzyme supporting our earlier suggestion that MGBG is acetylated by the nuclear N-acetyltransferase [8]. The inhibition of SAT by MGBG agrees with our results *in vitro* and also those of Pegg *et al.* [4] who found the drug to be a potent inducer *in vivo* and a weak inhibitor *in vitro*.

The purified enzyme had a similar subunit molecular weight to that purified from rat liver but it did not exhibit any cross reactivity in terms of immunoprecipitable protein. This may be a species difference or it could be an initial indication of the existence of a number of inducible isozymes of N-acetyltransferase, each of which responds to a different inducer. In this study MGBG was the inducing agent whereas in the rat liver carbon tetrachloride was used to induce the enzyme. Isozymes of polyamine degrading enzymes have been reported previously. Libby and Porter [16] observed two isoforms of polyamine oxidase in L1210 cells. Polyamine catabolism may therefore be regulated by the induction or utilization of different isozymes of the degrading enzymes. The choice of isozyme will then depend on the inhibitor of cell growth or toxic insult used.

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