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High specific induction of spermidine/spermine N^1-acetyltransferase in a human large cell lung carcinoma

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The cytotoxic response of the human large cell lung carcinoma line NCI H157 to exposure to the polyamine analogue N^1N^{12}-bis(ethyl)spermine (BESpm) is preceded by an extremely high induction of spermidine/spermine N^1-acetyltransferase (SSAT). The human enzyme has now been purified > 300-fold to apparent homogeneity and found to cross-react with antisera raised against rat liver SSAT. Although other acetylases are capable of acetylating polyamines using acetyl-CoA as the acetyl donor, the > 600-fold induction within 24 h was found to be specifically SSAT, since essentially all activity was precipitable by the specific antisera. The human enzyme appears to be similar to the rat enzyme in subunit size under reducing conditions (~ 20 kDa), substrate specificity and kinetic parameters. Preliminary results using actinomycin D and cycloheximide suggested that the unusually high induction by N^1N^{12}-bis(ethyl)spermine in the human lung cancer line result from new mRNA and protein synthesis. This hypothesis is further substantiated here by '*in vitro*' translation experiments comparing poly(A) mRNA from control and treated cells. The large cell lung carcinoma line NCI H157 represents a useful system to produce large amounts of the SSAT protein and to study the molecular events responsible for the induction and control of this important polyamine-metabolic enzyme. By using this rich source of SSAT protein, a partial amino acid sequence was determined by *N*-terminal sequencing of endoproteinase Lys-C digestion fragments. Further, this system should be useful in determining whether there is an association between the unusually high induction of the acetylase and the observed cytotoxicity in the NCI H157 cells.

INDUCTION

Recently, it has been found that bis(ethyl)polyamine analogues produce a pronounced cytotoxicity in the human large cell undifferentiated lung carcinoma line NCI H157 (Casero *et al.*, 1987, 1989*a*,*b*; Denstman *et al.*, 1987). Studies of the changes in the polyamine-metabolic pathway brought about by these agents have indicated that they lead to a very large increase in the ability of crude cell extracts to carry out the acetylation of spermidine (Casero *et al.*, 1989*a*,*b*). Treatment of NCI H157 cells with N^1N^{12}-bis(ethyl)spermine (BESpm) led to a more than 1000-fold rise in this activity. A number of enzymes are known to be able to catalyse the acetylation of polyamines using acetyl-CoA as an acetyl donor (Pegg, 1986; Kameji & Pegg, 1987). However, the results of the above studies are most consistent with the hypothesis that BESpm caused a specific induction of spermidine/spermine N^1-acetyltransferase (SSAT) activity. SSAT is a cytosolic enzyme which is known to be inducible in response to a variety of toxic agents, hormones and polyamine derivatives (Matsui *et al.*, 1981; Della Ragione & Pegg, 1982, 1983; Persson & Pegg, 1984; Bergeron *et al.*, 1984, 1988).

Although in some cases the extent of increase of SSAT has been more than two orders of magnitude, it has been very difficult to investigate the mechanism of this induction in detail, primarily because of the very small amount of SSAT actually present, even in the stimulated state. For example, SSAT activity was elevated by about 250-fold in the rat liver after treatment with carbon tetrachloride, but more than a 50000-fold purification was needed to obtain the homogeneous enzyme (Persson & Pegg, 1984).

The purpose of the present experiments was to determine if the rise in polyamine-acetylating capacity in the BESpm-treated human lung-cancer cells was indeed due specifically to increased SSAT activity, and to investigate the biochemical basis for this increase. It was found that there was an enormous increase in the content of SSAT protein in the NCI H157 cells and that these cells provided a convenient source for the purification and sequence determination of the human SSAT protein and for the investigation of the regulation of SSAT activity. Some properties of the human protein are described and compared with those of the purified rat protein.

MATERIALS AND METHODS

Materials

BESpm was synthesized by previously published methods (Bergeron *et al.*, 1982, 1984, 1988) and provided by the laboratory of Dr. Raymond J. Bergeron. [1-^{14}C]Acetyl-CoA (55 mCi/mmol) was purchased from ICN Radiochemicals, Irvine, CA, U.S.A. Silver staining of gels, protein determination and immunoblotting using the horseradish peroxidase method and 4-chloro-1-naphthol were carried out using reagents from Bio-Rad, Richmond, CA, U.S.A. Affi-Gel Blue and Hi-Pore 304 reversed-phase h.p.l.c. columns were also purchased from Bio-Rad. *sym*-Norspermidine–Sepharose was prepared as previously described (Della Ragione & Pegg, 1982). Other biochemical reagents were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A.

Assay of SSAT

SSAT activity was measured by monitoring the conversion of [1-^{14}C]acetyl-CoA into [^{14}C]acetylspermidine using an assay medium containing 100 mM-Tris/HCl, pH 7.8, 8 μM-[1^{14}C]acetyl-CoA and 3 mM-spermidine (Matsui *et al.*, 1981). Determination of K_m and $V_{max.}$ values for SSAT substrates was carried out as described by Della Ragione & Pegg (1983). Immunoprecipitation

Abbreviations used: BESpm, N^1N^{12}-bis(ethyl)spermine; SSAT, spermidine/spermine N^1-acetyltransferase.

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and immunoblotting of SSAT was carried out using a rabbit antiserum as previously described (Persson & Pegg, 1984). Purified rat liver SSAT was used as a marker protein.

Isolation and analysis of SSAT protein

Purification of rat liver SSAT was carried out by a modification of the procedure described by Della Ragione & Pegg (1982). $(NH_4)_2SO_4$-fractionated extracts were isolated from livers of carbon tetrachloride-treated rats and then applied to a column of DEAE-cellulose (15 cm × 5 cm) equilibrated with 50 mM-Tris (pH 7.5)/2.5 mM-dithiothreitol/0.1 mM-EDTA/0.02% Brij (buffer A) at a flow rate of 95 ml/h. The column was then washed with 500 ml of buffer A containing 0.1 M-NaCl and then eluted with 1500 ml of buffer A containing 1.5 M-NaCl. Fractions (10 ml) were collected and the tubes containing SSAT pooled and diluted with an equal volume of buffer A to reduce the NaCl concentration to 0.75 M. These diluted fractions were then applied at a flow rate of 40 ml/h to a column of norspermidine–Sepharose (6 cm × 2.5 cm) equilibrated with buffer A. The column was washed with 400 ml of buffer A containing 0.75 M-NaCl at 40 ml/h. The fractions with activity were pooled and concentrated to a volume of 10–20 ml in buffer A containing 1 mM-spermidine by repeated ultrafiltration using an Amicon YM-10 membrane. At least five cycles of 10-fold concentration and dilution with buffer A containing 1 mM-spermidine were used to remove the *sym*-norspermidine. The final sample was then purified on an Affi-Gel Blue column as previously described (Della Ragione & Pegg, 1982). The enzyme was stored frozen at −70 °C in aliquots to avoid freezing and thawing. The purity of the preparation was determined by polyacrylamide-gel electrophoresis under denaturing conditions using a 12.5% (w/v) polyacrylamide gel, which was fixed and stained using the silver-staining kit from Bio-Rad according to the manufacturer's instructions.

The NCI H157 line of human large cell lung carcinoma was used as the source of human SSAT protein. These cells were maintained before induction by serial passage as previously described (Casero *et al.*, 1989*b*). The human SSAT was purified from extracts of NCI H157 cells which had been exposed to 10 μM-BESpm for 24 h. The cell pellets were lysed by two freeze–thaw cycles using liquid N_2 and water at room temperature, followed by centrifugation at 17000 *g* for 20 min. The supernatant was then fractioned with $(NH_4)_2SO_4$ and chromatography on columns of DEAE-cellulose (8 cm × 2.5 cm), and norspermidine–Sepharose (14 cm × 2.5 cm) and Affi-Gel Blue (9 cm × 1 cm), similarly to the rat liver enzyme as above, except that the size of the columns was changed as indicated. The human SSAT protein was further purified by h.p.l.c. before determination of amino acid composition, sequencing and digestion with proteinases. It was applied to a Hi-Pore 304 reverse-phase column which had been washed with acetonitrile/water/trifluoroacetic acid (800:199:1, by vol.; solution B) and then equilibrated with water containing 0.1% trifluoroacetic acid (solution C). The sample was loaded and after 5 min a gradient of 0% solution B/100% solution C to 100% solution B/0% solution C over a period of 60 min at a flow rate of 1.5 ml/min was used. Peaks were detected by using a Beckman 163 variable-wavelength detector at 220 or 280 nm, collected, rechromatographed to check the purity and then used for amino acid composition or sequencing. The SSAT protein was digested with endoproteinase Lys-C for 4 h at 37 °C in 25 M-Tris/HCl, pH 8.5, containing 1 mM-EDTA, using 1 μg of proteinase/20 μg of protein. The resulting peptides were separated by h.p.l.c. as described above.

Amino acid analysis and sequence determination

Automated *N*-terminal sequence analysis of peptides was performed on a model 470A protein sequencer (Applied Biosystems). A portion (3 mg) of BioBrene Plus (Applied Biosystems) was applied on to the trifluoroacetic acid-etched glass-fibre filter and subjected to three cycles of Edman degradation before sample application. Conversion of the thiazolinone derivatives into phenylthiohydantoin amino acids was carried out with 25% (w/v) trifluoroacetic acid. Amino acid phenylthiohydantoin derivatives were separated by reverse-phase h.p.l.c. on a PTH C_{18} column (2.1 mm × 220 mm; 5 μm particle size; Applied Biosystems) with a sodium acetate/trimethylamine buffer, containing a 5% tetrahydrofuran/acetonitrile gradient as outlined by Applied Biosystems, on-line on a model 120A PTH Analyzer (Applied Biosystems).

The amino acid composition was determined, exactly as previously reported by Garboczi *et al.* (1988), by using a Waters–Millipore PICO-TAG system.

Translation *in vitro*

Synthesis of SSAT protein by translation of mRNA from NCI H157 cells was carried out essentially as described by Kameji & Pegg (1987), using rabbit reticulocyte lysates. Poly(A)-containing RNA was prepared by the guanidinium thiocyanate procedure, followed by passage through spun columns of poly(dT)–cellulose (Okayama *et al.*, 1989). The protein corresponding to SSAT was precipitated by using anti-SSAT antiserum (Persson & Pegg, 1984), separated by SDS/PAGE and revealed by fluorography.

RESULTS

As previously reported (Casero *et al.*, 1989*a*,*b*), treatment of NCI H157 cells with BESpm greatly increased the amount of

Table 1. Immunoprecipitation of acetylase activity from human lung carcinoma H157 cells by antiserum to rat liver SSAT

The samples (0.1 ml) were incubated overnight at 4 °C with 2.5 μl of buffer alone, normal rabbit serum or antiserum to SSAT, as indicated, in the presence of 1% BSA, 0.02% Brij 35 and 1 mM-spermidine in a total volume of 0.11 ml. Bacterial Protein A adsorbent (50 μl) was then added, and the samples further incubated for 90 min at 4 °C. The solution was then centrifuged at 15000 *g* for 3 min and the acetylase activity measured in the supernatant. The SSAT activity (column 4) was obtained by subtracting the values of antiserum-treated samples (column 3) from the values of the control-serum-treated samples (column 2) divided by 0.82. The correction factor of 0.82 was used since only 82% of the human SSAT was precipitated by the specific antiserum.

Treatment	Acetylase activity (nmol/min per mg)			
	No serum treatment	Control-serum-treated	Anti-SSAT-serum-treated	SSAT activity (nmol/min per mg)
Control	0.8±0.1	0.7±0.2	0.3±0.1	0.5±0.1
BESpm-treated	357±6	324±12	75±3	304±22

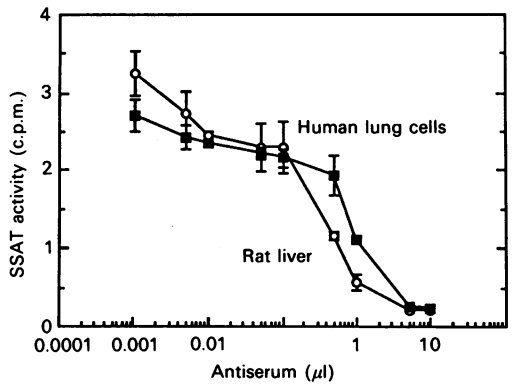

Fig. 1. Immunotitration of purified rat liver and purified NCI H157 lung-cancer cell spermidine/spermine N^1-acetyltransferase.

SSAT activity present in the cells (Table 1). The great majority of the induced activity was precipitable by an antiserum produced in rabbits to the purified rat liver SSAT, indicating that the activity is indeed SSAT (Table 1 and Fig. 1). The human SSAT appears to react slightly less well with the antiserum than the rat liver protein (Fig. 1), but the difference is quite small. The extent of induction of SSAT by BESpm amounted to approx. 600-fold at 24 h. This value is quite approximate, since the basal SSAT level was so low.

The increase in SSAT activity appears to be brought about by an increase in the amount of enzyme protein. A protein corresponding to an M_r of approx. 20000, somewhat larger than the purified rat liver SSAT ($\sim$ 18000), was detected on Western blots of crude extracts from BESpm-treated H157 cells developed with the antiserum to rat liver SSAT (Fig. 2). This band was not seen when extracts from control cells were used, so the precise extent of induction of SSAT protein cannot be determined directly. However, comparison of the intensity of the SSAT band in samples from the treated cells with that produced by a similar amount of activity of the purified rat liver protein suggested that the SSAT protein is induced to a very high level. SSAT was therefore purified from these cells using a protocol similar to that used for the rat liver enzyme (Table 2). As shown in Table 2, the initial specific activity of the extracts from the H157 cells was about 300 times greater than that of extracts from carbon tetrachloride-treated rat livers. The extracts were then subjected to precipitation with $(NH_4)_2SO_4$, batch elution from DEAE-cellulose, and followed by affinity chromatography on norspermidine–Sepharose and on Affi-Gel Blue. The final enzyme fraction gave a single band of M_r higher than the 18000-M_r marker, at $\sim$ 20000, when subjected to PAGE under denaturing conditions and developed with a silver stain (Fig. 3). The specific activity of this material was about three times that of the rat liver enzyme purified in the same way. This may represent a real difference between the human and the rat liver enzymes, since the rat liver preparation also gave a single band in the same analytical system (Fig. 3). However, confirmation of the observed difference is necessary, since the determination of protein may not be precise for the rat liver samples, from which only about 50 μg of protein was obtained after a 55000-fold purification.

As shown in Table 3, the properties of the human and the rat SSAT were very similar, except for the difference in specific activity described above. Of the natural polyamines, putrescine was not a substrate for either enzyme (results not shown), but both spermidine and spermine were aceylated. Spermidine exhibited higher K_m and $V_{max.}$ values. The K_m values for these substrates for the human enzyme were very similar to those found for the rat with spermidine and spermine.

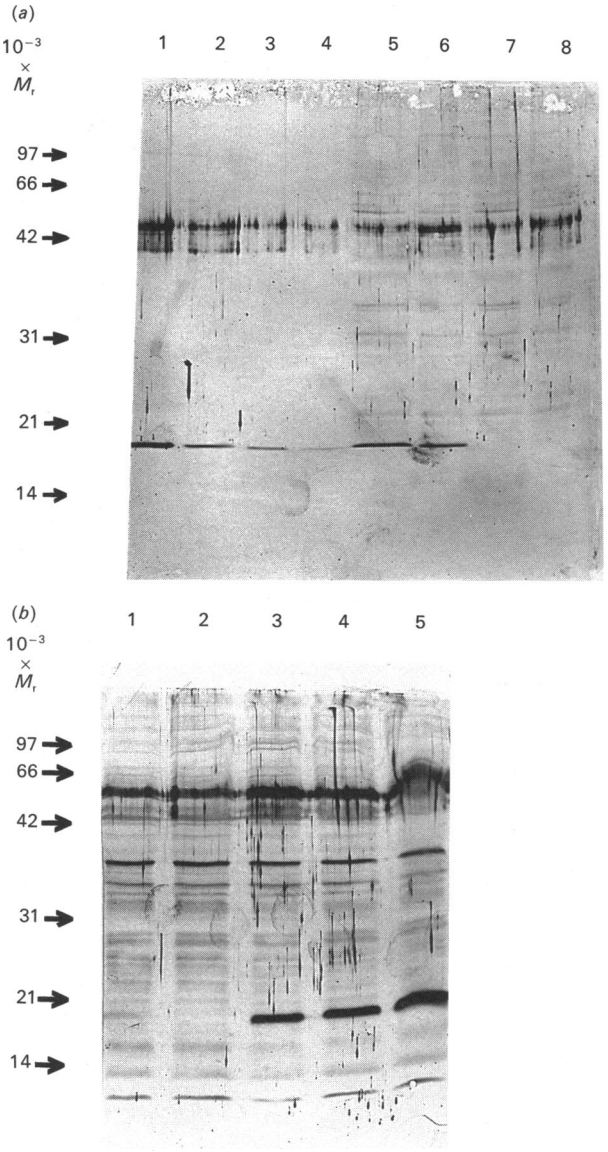

Fig. 2. Immunoblotting of extracts from BESM-treated NCI H157 cells using anti-spermidine/spermine N^1-acetyltransferase antiserum

Immunoblotting was carried out as described in the Materials and methods section. In (*a*), lanes 1–4 show results for purified rat livers SSAT (about 500, 200, 100 and 50 ng respectively); lanes 5 and 6 show results for extracts of NCI H157 cells treated with 10 μM-BESpm for 24 h (214 and 107 μg of protein respectively); and lanes 7 and 8 show results for extracts of untreated H157 cells (214 and 107 μg of protein respectively). The arrows indicate the positions of radioactive marker proteins of the M_r values indicated. The band of M_r about 50000 seen in all lanes was present in all samples, even those to which no extract was added and in those developed with a control antiserum not related to SSAT (results not shown). This band may represent a very strong reaction with an antibody directed against the bacterial proteins present in the Freund's adjuvant used for the immunizations. In (*b*) the lanes were loaded with extracts from untreated NCI H157 cells (lanes 1 and 2) and with extracts from cells treated with 10 μM-BESpm for 24 h (lanes 3–5, 128, 176, and 214 μg of protein respectively).

The human SSAT preparation was then subjected to reversed-phase h.p.l.c. analysis, and it was eluted as two sharp unresolved peaks. Attempts to obtain the *N*-terminal sequence from the purified human enzyme by automated gas-phase protein sequencing were unsuccessful, indicating that the *N*-terminus of the human enzyme is blocked. A portion of this purified protein

Table 2. Purification of SSAT from human lung carconoma H157 cells

Fraction	Protein* (mg)	Activity (units) (nmol/min)	Specific activity (nmol/min per mg)	Purification (fold)	Yield (%)
Cell supernatant	188	21896	116	1	100
$(NH_4)_2SO_4$ precipitate	49.1	10125	206	1.8	46
DEAE-cellulose	26.7	8254	309	2.7	38
Norspermidine–Sepharose	0.82	8865	10758	92	40
Affi-Gel Blue	0.12	4758	39000	335	22

* Represents typical recovery from the BESpm-treated NCI H157 cells.

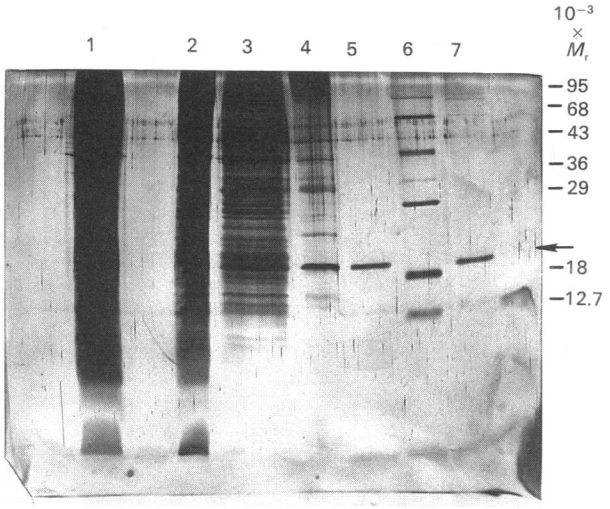

Fig. 3. Silver-stained gel of human SSAT after successive purification steps

Lane 1, crude extract; lane 2, 100 ng of $(NH_4)_2SO_4$ precipitates; lane 3, post-DEAE-cellulose column; lane 4, post-norspermidine-affinity column; lane 5, post-Affi-Gel Blue column; lane 6, silver-staining standards; lane 7, rat liver acetylase after Affi-Gel Blue chromatography. Each lane is loaded with protein amounting to an SSAT activity of 5.0 nmol/min (lane 3 has an activity of only 2.7 nmol/min). The arrow indicates SSAT.

Table 3. Comparison of human and rat SSAT

Property	SSAT... Rat	Human
Subunit M_r	18000	18000
$K_{m,spermidine}$ (mM)	0.13	0.07
$K_{m,spermine}$ (mM)	0.03	0.02
$V_{max.,spermidine}$ (μmol/min per mg)	9.5	38
$V_{max.,spermine}$ (μmol/min per mg)	2.1	9.9

was then used to determine amino acid composition (Table 4). On the basis of these data the endoproteinase Lys-C was chosen to produce reasonably sized peptide fragments for *N*-terminal sequence analysis. After repurification by h.p.l.c., the fragments designated C, D, E, I, J, and K in Fig. 4 were sequenced. Fragment peaks A and B were assumed not to be from the human SSAT protein, because they appeared in control samples where only the digestion buffer and the endoproteinase Lys-C were present. Table 5 lists the sequences determined for the designated peptide fragments.

Attempts also were made to sequence the peak designated L. However, nine out of the 15 attempted cycles yielded more than

Table 4. Amino acid analysis of human SSAT protein

The human SSAT protein was purified as indicated in the Materials and methods section from NCI H157 large cell lung carcinoma cells which were exposed to BESpm for 24 h.

Amino acid	Composition (%)
Asx	7.94
Glx	11.97
Ser	5.82
Gly	9.08
His	2.54
Arg	5.60
Thr	3.75
Ala	8.49
Pro	3.91
Tyr	3.91
Val	4.96
Met	2.67
Cys	1.15
Ile	8.09
Leu	9.57
Phe	5.02
Lys	5.53

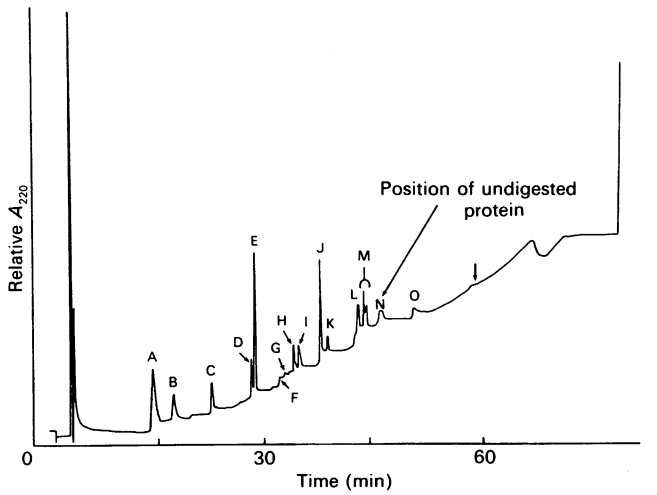

Fig. 4. Representative chromatograph of peptide fragment resulting from Lys-C digestion of the human SSAT protein

Purified human SSAT protein was subjected to Lys-C digestion and rechromatographed. The peaks designated A and B appeared in control digests containing only digestion buffer and the Lys-C endoproteinase; therefore these peaks were assumed not to result from the acetyltransferase, and no attempt was made to sequence them. Duplicate digestions and chromatography yielded identical results.

Table 5. Sequence of human SSAT Lys-C endoproteinase fragments

Peptide fragments were arbitrarily sequenced through a maximum of 15 or 25 cycles. Fragments C and D represent full-length sequences, since they end in a lysine residue. Where indicated, an 'X' represents a cycle which did not yield an unambiguous amino acid.

Peptide fragment	Position...	Sequence 5	10	15	20	25
C		E Y L L K				
D		X E Y M E E Q V I L T E K				
E		X R G A S D L S E E E G X R L				
I		X L S Q V A M R X R X S S M X F L V A E X N E P X				
J		X X W T P E G G S I V G F A M Y Y F T Y D P P I G				
K		X L Y L E X F F V M S D Y X X				

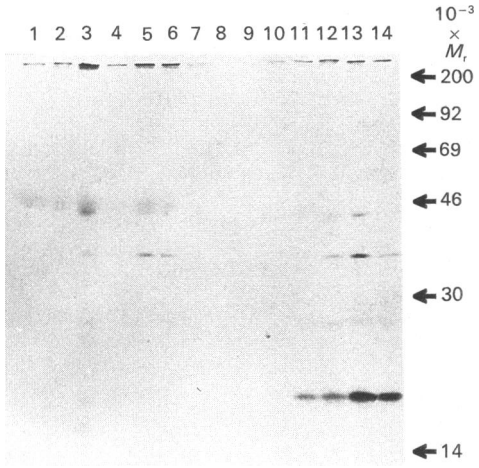

Fig. 5. Content of translatable SSAT mRNA in NCI H157 cells after treatment with 10 μm-BESpm

Poly(A)-containing RNA was isolated from NCI H157 cells and translated in a reticulocyte lysate in the presence of [^{35}S]methionine. The translation products were then precipitated with rabbit anti-SSAT and Protein A (lanes 9–14) or by a control rabbit serum and Protein A (lanes 1–7). The precipitates were solubilized and separated by SDS/PAGE and the labelled protein bands were developed by fluorography. The reaction mixtures used for lanes 1 and 9 contained no mRNA; all other reaction mixtures contained 1.5 μg of RNA from cells treated with 10 μM-BESpm for 0 h (lanes 2 and 10), 6 h (lanes 3 and 11), 12 h (lanes 4 and 12), 24 h (lanes 5 and 13) and 48 h (lanes 6 and 14). Lane 7 contained 1.5 μg of Chinese-hamster-ovary-cell mRNA, and lane 8 was not used. The arrows indicate the position of radioactive marker proteins with the M_r values indicated.

one amino acid, indicating a possible failure to separate peaks that were eluted close together. No attempts were made to sequence peaks F, G, and M because of their size and/or poor separation from neighbouring peaks.

Previous studies using actinomycin D as an inhibitor of mRNA synthesis indicated that the observed induction of SSAT by BESpm in the human lung-cancer cells may be due to specific increases in SSAT mRNA (Casero *et al.*, 1989*a*,*b*). In order to test more rigorously whether the increased accumulation of SSAT protein after BESpm treatment of NCI H157 cells is brought about by changes in the steady-state mRNA for SSAT, poly(A) RNA was isolated from cells after various periods of exposure to BESpm. This RNA was then translated in a reticulocyte lysate and the [^{35}S]methionine-labelled protein, recognized by rabbit anti-SSAT antiserum, was precipitated (Fig. 5). Translated poly(A) RNA from untreated cells produced no detectable levels of antiserum-recognizable protein. However, poly(A) RNA from the BESpm-treated NCI H157 cells produced time-dependent increases of antiserum-recognizable protein with an apparent M_r of ~ 20000, with a peak observed after 24 h exposure to 10 μM-BESpm.

DISCUSSION

The inducibility of SSAT by the polyamines and their analogues has been known for some time (Pegg, 1986; Della Ragione & Pegg, 1982, 1983). However, the mechanisms responsible for the observed induction are not fully understood. In the current work we have characterized the unusually high induction of SSAT in a human lung-cancer cell line in response to treatment with BESpm.

Through the use of specific rabbit antiserum raised against rat liver SSAT (Persson & Pegg, 1984), it was possible to confirm our previous findings, indicating that the observed increase in acetylase activity is, in fact, due to the specific induction of SSAT (Casero *et al.*, 1989*b*), since most of the induced activity was precipitable by the antiserum. The inability to precipitate as much of the SSAT activity as reported for the rat protein (Persson & Pegg, 1984) may be due to comparatively poorer recognition of the human protein by the anti-(rat SSAT) antiserum. However, it is clear from Western-blot and silver-stain analysis that the human SSAT has an M_r of ~ 20000, similar to the M_r of ~ 18000 previously observed for the purified rat and chicken proteins (Persson & Pegg, 1984; Shinki & Suda, 1989).

Kinetic studies clearly indicate that, in addition to subunit molecular mass, the human and the rat SSAT protein share many similarities. The only apparent significant difference exists in the specific activity of the enzymes. The human enzyme in these initial studies exhibits an approx. 3-fold greater specific activity than the purified rat protein. Although this difference may be significant, and partly responsible for the extreme induction of activity observed in the BESpm-treated human cell line, it will be necessary to confirm these differences. At least two possibilities exist for the observed difference in specific activities between rat and human. The rat liver protein required > 50000-fold purification; therefore it is possible that significant activity may have been lost during the purification process. Also, the specific-activity estimations were performed using a very limited amount of rat SSAT protein, which may have led to a underestimation of specific activity.

The large amounts of the SSAT protein available in the induced human lung-tumour line provided an excellent source for purification and use in the analysis of primary amino acid sequence information. Clearly, further study is necessary to determine the entire primary amino acid sequence. However, some general observations should be noted regarding the avail-

able data. Peptide fragment J represents a largely hydrophobic region composed of seven of 25 aromatic amino acids with a cluster of potentially reactive tyrosine residues with the structure YYFTY. Fragments D and E, however, both represent relatively hydrophilic peptides. It should also be noted that the sequences currently available will be valuable in the further study of the SSAT protein, especially in the verification of any future cloned sequences putatively corresponding to the SSAT gene. Particularly useful in this regard is the sequence EYMEEQV found in fragment D. This sequence can be used to generate an oligomer of 20 nucleotides with only a 32-fold redundancy, which should be useful in the screening of cDNA libraries for a cDNA corresponding to the SSAT protein.

Results from previous studies are consistent with the hypothesis that the observed induction of SSAT activity is due to the synthesis of new protein, which in turn results from newly synthesized mRNA (Casero *et al.*, 1989*b*; Shinki *et al.*, 1985; Libby *et al.*, 1989). Our current findings, using an '*in vitro*' translation system, are also consistent with the basis for enzyme induction being related to new transcription of message coding for the SSAT protein, followed by new protein synthesis. That the polyamines and their analogues may effect the regulation of gene expression has been documented in other systems (Celano *et al.*, 1989*a*,*b*; Moore *et al.*, 1988; Watanabe *et al.*, 1986). We and others have shown that changes in intracellular polyamine concentrations can significantly and specifically change the transcription rate of several important growth-related genes, most notably the c-*myc* oncogene (Celano *et al.*, 1989*a*,*b*).

Libby *et al.* (1989) have recently reported that L1210 murine leukaemia cells also respond to exposure to the bis(ethyl)-polyamines with an induction of SSAT. Although the absolute levels of induction are considerably less than reported here, their results similarly indicate that the observed induction is due almost entirely to new protein synthesis, a portion of which is probably due to new mRNA synthesis. Similarly, Shinki *et al.* (1985) reported that the induction of SSAT by 1,25-dihydroxy-vitamin D_3 was greatly reduced by prior treatment with cyclohexamide or actinomycin D. It is also noteworthy that the content of polyamines and/or their analogues may have a direct effect on gene expression at the level of translation. As previously shown (Kameji & Pegg, 1987; Pegg *et al.*, 1988), the translation of mRNAs for both ODC and AdoMetDC is profoundly affected by the concentration of polyamines or their analogues in an '*in vitro*' reticulocyte translation assay. It is therefore possible that some of the large induction of SSAT observed here may result from changes in the translation rate of the mRNA coding for the acetylase protein. However, final confirmation of these points regarding the expression of SSAT will require the availability of cloned sequences of the human SSAT gene.

It is important to note that the NCI H157 line of human large cell undifferentiated carcinoma represents an extremely sensitive tumour line to BESpm treatment (Casero *et al.*, 1989*a*,*b*), responding in a rapid, cytotoxic, manner. Additionally, this line responds with the greatest amount of SSAT induction observed thus far in any system (Casero *et al.*, 1989*b*). Most human and murine tumour and normal cells do not respond to the bis(ethyl)-polyamine analogues, or other compounds, with a similar induction of SSAT (Persson & Pegg, 1984; Libby *et al.*, 1989; Pegg *et al.*, 1985; Erwin & Pegg, 1986), and do not respond in a cytotoxic manner to the bis(ethyl)polyamines. The large-cell system may therefore represent both an excellent system for the study of the basic mechanisms of the regulation of the SSAT gene and a potential aid in the understanding of the unusual sensitivity of the large-cell phenotype to treatment with the bis(ethyl)polyamines.

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