Combined regulation of ornithine and *S*-adenosylmethionine decarboxylases by spermine and the spermine analogue *N*¹*N*¹²-bis(ethyl)spermine

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In the present study, the spermine (SPM) analogue *N*¹*N*¹²-bis(ethyl)spermine (BESPM) is compared with SPM in its ability to regulate ornithine decarboxylase (ODC) and *S*-adenosyl-L-methionine decarboxylase (AdoMetDC) activities in intact L1210 cells and in the mechanism(s) by which this is accomplished. Unlike the comparable spermidine (SPD) analogue *N*¹*N*⁸-bis(ethyl)spermidine, which regulates only ODC, BESPM suppresses both ODC and AdoMetDC activities. With 1 μ M-SPM or -BESPM, near-maximal suppression of enzyme activity (i.e. <70 %) was achieved after 2 h for ODC and 12 h for AdoMetDC. After such treatment, ODC activity fully recovered within 2–4 h, and that of AdoMetDC within 12 h, when cells were reseeded into drug-free media. It was deduced that an intracellular accumulation of BESPM or SPM equivalent to only ~200–450 pmol/10⁶ cells was sufficient to fully invoke ODC regulatory mechanisms. Decreases in both enzyme activities after BESPM or SPM treatment were closely paralleled by concomitant decreases in the amount of enzyme protein. Since cellular ODC or AdoMetDC mRNA was not similarly decreased by either BESPM or SPM treatment, it was concluded that translational and/or post-translational mechanisms were probably responsible for enzyme regulation. In support of the former of these possibilities, it was demonstrated that both BESPM and SPM preferentially inhibited the translation *in vitro* of ODC and AdoMetDC relative to albumin in a reticulocyte-lysate system. On the basis of the consistent similarities between BESPM and SPM in all parameters studied, it is concluded that the analogue most likely acts by mechanisms identical with those by which SPM acts in suppressing polyamine biosynthesis.

INTRODUCTION

Perhaps, in accordance with the essential role of polyamines in proliferative responses, cells have evolved sensitive mechanisms to regulate polyamine biosynthesis and to maintain intracellular pool sizes. In addition to those involving uptake, the best known are those which control the levels of the two substrate lead-in enzymes of the pathway, namely ornithine carboxylase (ODC) and *S*-adenosylmethionine decarboxylase (AdoMetDC) respectively. Thus decreases in polyamine pools, such as those invoked by enzyme inhibitors, result in substantial rises in one or both of these enzyme activities (Pegg, 1988). Contrariwise, increases in polyamine pools, such as those produced by exposure of cells to exogenous polyamines, rapidly and dramatically lower ODC and AdoMetDC activities.

As an alternative antiproliferative approach to the use of inhibitors directed at polyamine-biosynthetic enzymes, we have, for some time, been developing a strategy based on analogue exploitation of pathway-regulatory mechanisms (Porter & Sufrin, 1986; Porter & Bergeron, 1988*a,b*). Specifically, polyamine analogues were synthesized and selected for their ability to regulate negatively ODC and/or AdoMetDC and, at the same time, for their inability to substitute for the natural polyamines in functions related to cell growth.

Recently, we demonstrated that the spermidine (SPD) analogue best fitted to these criteria, namely *N*¹*N*⁸-bis(ethyl)spermidine (BES), suppressed ODC activity

(but not AdoMetDC activity) via mechanisms which coincided with those utilized by SPD itself (Porter *et al.*, 1987*a*). At intracellular concentrations in the order of 30 % of the total SPD pool, both exogenous SPD and BES evoked post-transcriptional control mechanisms which effectively depleted cells of ODC activity (Porter *et al.*, 1987*a*). Specifically, within 2 h of treatment, ODC activity and protein were diminished by at least 80 %, whereas ODC mRNA was only marginally affected. In α -difluoromethylornithine (DFMO)-resistant L1210 cells, it was shown that BES decreased ODC synthesis and concomitantly increased enzyme degradation (Pegg *et al.*, 1988).

Subsequently we found that, whereas BES reduced only ODC activity, the comparable spermine (SPM) analogue *N*¹*N*¹²-bis(ethyl)spermine (BESPM, Fig. 1), concomitantly decreased both ODC and AdoMetDC activities and was approx. 30 times more effective, on a concentration basis, in inhibiting cell growth (Porter *et al.*, 1987*a,b*). It thus became of interest to compare the regulatory control of AdoMetDC by BESPM with that already known for ODC (Pegg *et al.*, 1988). Since BESPM has demonstrated meaningful antiproliferative activity with particular potency against human large-cell lung carcinoma (Casero *et al.*, 1989) and melanoma lines (Porter *et al.*, 1988; Libby *et al.*, 1989), it and related analogues (Bergeron *et al.*, 1989) have shown promise for continued development towards clinical trial. In support of these efforts and to facilitate the identification of

Abbreviations used: AdoMet, *S*-adenosyl-L-methionine; AdoMetDC, AdoMet decarboxylase; BES, *N*¹*N*⁸-bis(ethyl)spermidine; BESPM, *N*¹*N*¹²-bis(ethyl)spermine; DFMO, α -difluoromethylornithine; ODC, ornithine decarboxylase; PBS, phosphate-buffered saline (0.8 % NaCl/0.115 % Na₂HPO₄/0.02 % KH₂PO₄/2H₂O/0.02 % KCl, pH 7.5); RPMI-1640, Roswell Park Memorial Institute Medium 1640; SPD, spermidine; SPM, spermine; IC₅₀, concentration causing 50 % of maximal inhibition.

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Fig. 1. Structural representations of the natural polyamine, SPM, and the SPM analogue N^1N^{12} -bis(ethyl)spermine (BESPM)

additional analogues, we have undertaken to characterize and compare regulation of the decarboxylases by BESPM and SPM.

MATERIALS AND METHODS

Materials

BESPM was synthesized as a hydrochloride salt as described elsewhere (Bergeron *et al.*, 1988). [^{14}C]SPM was obtained from New England Nuclear Corp. (Boston, MA, U.S.A.). The cDNA (pODC 934) encoding mouse kidney ODC was kindly provided by Dr. Franklin G. Berger (Berger *et al.*, 1984). A pBR322 plasmid containing a cDNA insert for rat AdoMetDC, pSAM.1, was prepared as described elsewhere (Pegg *et al.*, 1987; Pajunen *et al.*, 1988).

Cell culture

Murine L1210 cells were grown as a suspension culture in RPMI-1640 medium containing 2% (w/v) Hepes/Mops buffer, 1 mM-aminoguanidine as an inhibitor of serum diamine oxidase and 10% (v/v) Nu-Serum IV (Collaborative Research, Lexington, MA, U.S.A.) as a semi-defined serum substitute. Cells were seeded in 75 cm² plastic tissue-culture flasks and allowed to grow for 16 h before addition of 1 μM -BESPM or -SPM. The seeding inoculum was adjusted so that the cell density at treatment was approx. 3×10^5 cells/ml. At 1–24 h afterwards, cells were removed and washed for subsequent analysis.

For recovery studies, cells were treated as described above for 12 h with 1 μM -BESPM or radiolabelled SPM, washed once with warm PBS and reseeded in drug-free medium at 1.5×10^5 cells/ml. Cells were then harvested at 1–12 h afterward for subsequent analysis.

For serum-stimulation studies, cells were cultured for 72 h at plateau phase and then reseeded in fresh warm medium in the presence or absence of 1 μM -BESPM or -SPM, or cells were harvested at 2–48 h afterwards. Those under treatment with BESPM or SPM were analysed at 4 and 24 h for decarboxylase activities.

Decarboxylase activity and protein

Extracts for measurement of enzyme activity and protein were prepared by sonicating control or treated cells in 10 mM-Tris/HCl, pH 7.2, containing 0.5 mM- Na_2EDTA , 5 mM-dithiothreitol and 50 μM -pyridoxal 5'-phosphate, followed by centrifugation at 20000 *g* for 20 min. Decarboxylase activities were determined by measuring the release of $^{14}\text{CO}_2$ from L-1-[^{14}C]substrate in accordance with the methods of Pegg & Seely (1983) and Pegg & Pösö (1983). Enzyme protein was quantified by radioimmunoassay as described elsewhere, using monospecific rabbit antisera to mouse ODC or AdoMetDC (Shirahata *et al.*, 1985; Porter *et al.*, 1987a).

Analysis of ODC mRNA

Total RNA was isolated by the guanidinium chloride method (Cox, 1968). To measure ODC mRNA, 10 μg of RNA was

fractionated on 1.5% formaldehyde/agarose gels, transferred to nitrocellulose and hybridized to ^{32}P -labelled pODC934 DNA, a plasmid which contains sequences complementary to mouse kidney ODC mRNA (Berger *et al.*, 1984) or a labelled *Eco*R1 fragment of rat AdoMet cDNA (Pajunen *et al.*, 1988). Hybridization was observed after autoradiography.

Intracellular polyamines

A sample of 10^7 cells was taken for polyamine determinations and extracted with 0.6 M- HClO_4 . The extract was analysed by h.p.l.c. with a system based on cation exchange and post-column derivatization with *o*-phthaldehyde as described elsewhere (Porter *et al.*, 1985).

Intracellular BESPM

Because BESPM lacks primary amino groups and is, therefore, minimally reactive with *o*-phthaldehyde (Benson & Hare, 1975), intracellular concentrations of the analogues were measured by h.p.l.c. after pre-column derivatization with dansyl chloride by using a polyamine-analysis system similar to that described elsewhere (Kabra *et al.*, 1986).

Effect of BES and BESPM on translation of mRNA for AdoMetDC

The effects of polyamines and analogues on the synthesis of labelled AdoMetDC protein in reticulocyte lysates was carried out as described by Kameji & Pegg (1987a,b). Poly(A)-containing RNA was isolated from prostates of rats treated with DFMO to increase the content of AdoMetDC mRNA and used in the translation reaction mixtures, which contained 0.5 μg of this RNA. In addition, 0.05 μg of rat liver mRNA was added to provide a source of albumin synthesis (Kameji & Pegg, 1987a). The synthesis of albumin and of AdoMetDC were determined by using specific antisera for each of these proteins to precipitate the relevant translation products, which were then separated by PAGE. The protein bands were revealed by fluorography, and the amounts were quantified by densitometric scanning of the films. The AdoMetDC values were obtained by adding the sum of the 38000- M_r proenzyme and the 32000- M_r enzyme subunit bands (Kameji & Pegg, 1987b). The polyamines added did not change the relative proportions of these two products.

RESULTS

On the basis of previous studies (Porter *et al.*, 1987a) in which 3 μM was found to be the minimal effective concentration suppression of ODC activity by SPD or BES, and on considerations related to relative IC_{50} (concentration causing 50% of maximal inhibition) values (Porter *et al.*, 1987a), 1 μM was selected as the working concentration for the present studies with BESPM and SPM. The concentration is well below IC_{50} values and is completely non-toxic for either drug during incubations up to 24 h. In cells pre-seeded for 16 h before treatment and growing at the time of treatment under steady-state conditions, 1 μM -SPM or -BESPM rapidly suppressed ODC activity, reaching a maximal effect of $\sim 80\%$ decrease by 1–2 h. Thereafter enzyme activity remained suppressed in the presence of the drugs. Removal of either drug resulted in a rapid recovery in enzyme activity, which was complete by 4 h in cells treated for either 2 h (Table 1) or 12 h (Fig. 2). If in the case of the former, cells were treated with either [^{14}C]SPM or BESPM and assayed for drug content under conditions of enzyme suppression and enzyme recovery, the approximate amount of drug required to achieve ODC suppression could be deduced (Table 1). Thus, since ODC is fully recovered in cells containing an amount of SPM or BESPM equivalent to $\sim 7\%$ of the total SPD plus SPM

Table 1. Intracellular polyamine pools under defined conditions of suppression and recovery of ODC activity by BESPM or [¹⁴C]SPM

Abbreviation used: PUT, putrescine; n.d., not determined.

Conditions	ODC activity (% of control)	Size (pmol/10 ⁶ cells)				
		Polyamine pools*			BESPM or [¹⁴ C]SPM†	% of control SPD + SPM pool
		PUT	SPD	SPM		
2 h treatment						
Control	100	200	2830	540	—	—
1 μ M-BESPM	16	n.d.	2650	750	395	12
1 μ M-[¹⁴ C]SPM	21	n.d.	2555	515	460	14
2 h treatment, 4 h recovery						
Control	100	280	1865	395	—	—
1 μ M-BESPM	120	240	1590	495	195	6
1 μ M-[¹⁴ C]SPM	110	170	1410	420	265	8

* Data from two experiments performed in duplicate; in all cases, variation in polyamine pools relative to control cells was less than 10 % between experiments.

† Of the total cellular radioactivity, 98 % was found to be present as SPM and only 2 % as SPD.

Fig. 2. Kinetics of ODC (a) and AdoMetDC (b) suppression by 1 μ M-SPM (i) or BESPM (ii)

Cells were grown continuously for 1–24 h in the presence of either drug (continuous line) or reseeded at 12 h into drug-free medium (broken line). Control ODC and AdoMetDC activities averaged 9.9 and 2.1 nmol/h mg protein respectively.

pools and fully suppressed in cells containing ~13 % of those same pools, the minimum amount required to suppress the enzyme is between 7 and 13 % of the combined SPD and SPM pool.

In contrast with ODC, suppression of AdoMetDC activity occurred much more slowly and did not achieve a maximal effect until 12 h, when the activity was approx. 30 % of control for both BESPM- and SPM-treated cells. Initially the decline in

activity occurred rapidly during the first 1–2 h, but became more gradual thereafter (Fig. 2). The effect was comparable for both SPM and BESPM, suggesting the involvement of the similar mechanisms. When, following treatment for 12 h with either 1 μ M-SPM or -BESPM, cells were reseeded into drug-free medium (Fig. 2), AdoMetDC activity recovered to control levels within 12 h, a period considerably longer than the 2 h required for ODC. Experiments comparable with those performed in deter-

Table 2. Effect of SPM and BESPM on the ODC and AdoMetDC activity, protein and mRNA content of L1210 cells

Conditions	ODC		AdoMetDC	
	Activity/protein* (units/ng)	mRNA† (units/μg)	Activity/protein* (units/ng)	mRNA† (units/ug)
12 h treatment				
Control	1.8	1.00	0.72	1.00
1 μM-SPM	2.2	1.10	0.75	0.91
1 μM-BESPM	1.8	1.08	0.71	0.77
12 h treatment, 12 h recovery				
Control	1.2	1.00	0.81	1.00
1 μM-SPM	1.3	1.30	0.81	1.26
1 μM-BESPM	1.3	1.37	0.76	1.31

* The content of enzyme protein was determined by radioimmunoassay; results are means for duplicate samples with agreement within $\pm 10\%$.

† Quantified by densitometric scanning of dot-blot preparations.

Fig. 3. Effect of increasing concentrations of SPM (a) or BESPM (b) on the translation *in vitro* of AdoMetDC, albumin and total protein in the reticulocyte lysate system

Results represent the means of three determinations with agreement within $\pm 15\%$.

mining the minimum drug concentration required for ODC suppression were not possible with AdoMetDC, because of the prolonged treatment period. However, from the similarity in initial decline in AdoMetDC to that of ODC, it would appear that a comparable concentration of SPM or BESPM (7–13%) is also sufficient to initiate regulatory mechanisms for AdoMetDC.

Taking treatment with 1 μM-BESPM or -SPM for 12 h as the minimum condition for suppressing both enzyme activities, the

effects on enzyme protein and mRNA were also characterized (Table 2). With both ODC and AdoMetDC, the decrease in enzyme activities was accompanied by a comparable fall in immunoprecipitable enzyme protein, confirming that regulatory mechanisms, as opposed to direct enzyme inhibition, was involved. Likewise, an increase in enzyme activity during recovery in drug-free medium was also accompanied by an increase in enzyme protein. Quantification of enzyme mRNA in identically treated cells revealed a constancy in both ODC and AdoMetDC mRNA levels under all conditions of treatment and recovery (Table 2). Thus BESPM and SPM appear to behave very similarly in suppressing both ODC and AdoMetDC via post-transcriptional regulatory mechanisms.

In consideration of previous studies indicating that the post-transcriptional mechanisms might involve inhibition of enzyme-protein translation (Kameji & Pegg, 1987a; Pegg *et al.*, 1988; Holm *et al.*, 1989; Persson *et al.*, 1989), the relative effects of SPM and BESPM on the translation of AdoMetDC, albumin and total protein *in vitro* were examined using a reticulocyte-lysate system. At concentrations readily achievable in cells (i.e. 50–100 μM), both SPM and BESPM selectively reduced AdoMetDC translation while minimally inhibiting albumin or total protein synthesis (Fig. 3). We have previously noted (Porter *et al.*, 1987b), that, unlike BESPM, BES at concentrations which negatively regulate ODC in cells have only a minimal effect on AdoMetDC activity. Thus it was of interest to determine whether a similar differential in enzyme effects existed in the '*in vitro*' translation system. In agreement with 'cellular' findings, BES and SPD inhibited AdoMetDC translation, but at concentrations 10-fold higher than those required for BESPM and SPM (Fig. 4). As with BESPM, the effects were selective for AdoMetDC when compared with total protein or albumin. The effects of BES and BESPM on ODC translation have been previously reported (Pegg *et al.*, 1988).

Finally, it became of interest to determine whether SPM and BESPM can prevent the induction of ODC and AdoMetDC in cell cultures maintained at plateau phase and then reseeded into fresh medium. Characterization of the kinetics of enzyme induction revealed peaks of ODC and AdoMetDC activities at approx. 4 h after reseeded (Fig. 5). This time point was adopted for subsequent enzyme-regulation studies. The inclusion of 1 μM-SPM or -BESPM in the reseeded medium only partially prevented this rise in enzyme activity (Table 3). Whereas in control cells ODC increased 8.2-fold and AdoMetDC 2.9-fold, ODC rose 3.7-fold and AdoMetDC 2.0-fold in BESPM-treated cells. SPM was similar to BESPM in suppressing enzyme activities. Thus it would appear that exogenous SPM and BESPM are

Table 3. Suppression of ODC and AdoMetDC induction by BESPM or SPM in cultured L1210 cells after reseeding

Culture	Treatment	ODC		AdoMetDC	
		Activity (nmol/h per mg)	Fold increase*	Activity (nmol/h per mg)	Fold increase*
Plateau 72 h	None	1.70	—	1.29	—
Reseeded 4 h	None	13.91	8.2	3.82	3.0
	1 μ M-SPM	8.61	5.1	2.46	1.9
	1 μ M-BESPM	6.35	3.7	2.59	2.0

* Over cells maintained at plateau phase for 72 h.

Fig. 4. Effect of increasing concentrations of SPD (a) or BES (b) on the translation *in vitro* of AdoMetDC, albumin and total protein in the reticulocyte lysate system

Results represent the means of three determinations which agreed within $\pm 15\%$.

somewhat more effective in suppressing steady-state ODC and AdoMetDC than they are in preventing growth-related increases in enzyme activity.

DISCUSSION

The finding that ODC activity and protein decline in response to exogenous SPM or BESPM, whereas levels of ODC mRNA do not, strongly suggests that regulatory control of this enzyme by either SPM or the analogue is exerted via post-transcriptional mechanisms. Except for being effective at slightly lower extracellular concentrations, BESPM behaves identically with the SPD analogue BES in this regard, as demonstrated in both wild-type (Porter *et al.*, 1987a) and DFMO-resistant L1210 cells (Pegg *et al.*, 1988). Because the latter contain very high levels of ODC,

Fig. 5. Increases in ODC (●) and AdoMetDC (○) activities in L1210 cells maintained 72 h at plateau phase and then reseeded into fresh medium and allowed to grow for up to 24 h

Note that both enzyme activities peak initially at approx. 4 h after reseeded, the point chosen for enzyme-suppression studies with SPM and BESPM (Table 3). Results are means for two experiments exhibiting agreement within $\pm 10\%$.

it has been possible to show that, in addition to affecting enzyme synthesis, the analogues or their physiological counterparts also increase degradation of ODC.

A distinction between the SPM analogue BESPM and the SPD analogue BES, which has been previously indicated (Porter *et al.*, 1987b), is that, at concentrations which fully suppress ODC, BESPM also suppresses AdoMetDC activity, whereas BES does not. Thus, in studying BESPM effects on ODC, it is possible to compare regulation of AdoMetDC. The substantial differences in the kinetics of enzyme response (Fig. 2) may simply reflect the different half-life values for the two decarboxylases, since other differences were not detectable insofar as these were compared. Like ODC protein, AdoMetDC protein declined in parallel with activity, whereas mRNA levels remained high, suggesting ODC-like post-transcriptional mechanisms such as inhibition of protein translation and/or increased enzyme degradation (Pegg *et al.*, 1988). Interestingly, these mechanisms seem to be most effective during steady-state growth conditions. The relative inability of the analogues to prevent the rise in decarboxylase activities associated with culture reseeded (Table 3) is unexpected, since post-transcriptional mechanisms should override increases in enzyme activity that are known to result from transcriptional activation under such conditions (Katz & Kahana, 1987; Sertich & Pegg, 1987).

Since wild-type L1210 cells do not contain sufficient enzyme protein to examine reliably the latter, an '*in vitro*' system was used to study the effects of analogue on translation. The results

provide clear evidence for the potential of either SPM or BESPM to inhibit selectively ODC or AdoMetDC synthesis in intact cells. In the case of ODC, the minimum effective concentration of BESPM required to inhibit enzyme translation (i.e. 75–100 μM , Fig. 3; Pegg *et al.*, 1988) is actually slightly less than the minimum intracellular amount required to initiate and sustain enzyme suppression [i.e. 200–300 μM (Table 1) determined using previously obtained cell-volume estimates (Pera *et al.*, 1987a)]. Further support for the role of translational control derives from the correlation between the abilities of BES and BESPM to inhibit AdoMetDC translation *in vitro* and their relative effects on this enzyme in cells (Porter & Sufrin 1986). In both cases, BESPM is much more effective than BES. Likewise, the finding that putrescine is relatively ineffective in inhibiting ODC translation (Pegg *et al.*, 1988; Holm *et al.*, 1989) agrees with our previous results that N^1N^5 -bis(ethyl)putrescine (which cannot be converted by cells into SPD or an SPD analogue) is only weakly able to influence ODC activity in cells (Porter *et al.*, 1987b). Taken together, these correlations provide support for the validity of the '*in vitro*' translational system as an indicator of cellular events.

The exact mechanism(s) by which the natural polyamines interfere with decarboxylase synthesis are not well understood. Initially, it was considered that the effect may involve polyamine binding at the unusually long non-coding 5'-leader sequence of the ODC mRNA (Gupta & Coffino, 1985; Katz & Kahana, 1988), producing a change in secondary structure and hence a decrease in the rate of initiation. However, deletion of most of the 5'-non-translated sequence did not prevent polyamine regulation of ODC mRNA translation (Pegg *et al.*, 1988). Further, in examining the polysome profiles of polyamine-depleted cells, Holm *et al.* (1989) found that the resulting increased enzyme synthesis was not due to increased initiation or rate of elongation, since there were no significant shifts in the ODC mRNA towards larger polysomes. If this also applies to conditions of polyamine excess, the action of polyamines on ODC and AdoMetDC translation may involve more subtle interactions with ribosomal proteins and/or mRNA.

If the apparently greater sensitivity of AdoMetDC control mechanisms to BES than to BESPM is also true for their natural counterparts, the phenomenon may have important metabolic implications. Owing to the rapid metabolic conversion of exogenously supplied SPD into SPM, this distinction is difficult to demonstrate with the natural polyamines. Yet our findings with the '*in vitro*' translation system suggest that it may exist, since about 8-fold more SPD than SPM was required to comparably affect AdoMetDC synthesis. Such differential control of AdoMetDC by SPM could contribute to the cell's ability to maintain appropriate metabolic balances between SPD and SPM synthesis and pools. The alternative means, via SPM synthase, is unlikely, since the enzyme usually exists in excess of the decarboxylases, has a relatively long-life and is insensitive to regulatory controls (Pegg, 1986; Persson *et al.*, 1988). The selective regulation of the decarboxylases also increases the possibility of identifying analogues more potent than the polyamines themselves in this activity (Porter *et al.*, 1988), and apparent structure-function relationships are beginning to emerge. Indeed, in contrast with BES, which can be used to regulate only ODC, we have recently identified an unsaturated SPM analogue that regulates AdoMetDC but not ODC (Porter *et al.*, 1988).

The finding that BESPM is mechanistically similar to, and almost identically efficient with, SPM in suppressing enzyme activity in growing cells reinforces the potential of this approach as an antiproliferative strategy. Likewise, it is noteworthy that

the analogues were found partially to negate the peaks in decarboxylase activities associated with the resumption of growth in media-fed cells and to prevent the compensatory rise in enzyme activities associated with the use of enzyme inhibitors (Porter *et al.*, 1988). Both activities have chemotherapeutic implications.

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