Aliphatic Chain Length Specificity of the Polyamine Transport System in Ascites L1210 Leukemia Cells

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ABSTRACT

A series of diamine homologues of putrescine and triamine homologues of spermidine was used to determine the structural specificity of the polyamine transport system in ascites L1210 leukemia cells by measuring their ability to compete with [3H]-putrescine, [3H]-spermidine, or [3H]-spermine for uptake. Transport specificity among the diamines (as indicated by K_m constants) was greatest for those having chain lengths similar to that of spermidine and least for those similar to putrescine. Among the triamines, transport specificity was greatest for those having an overall chain length similar to those of spermidine and spermine. The homologue competition profiles were relatively the same for [3H]-putrescine, [3H]-spermidine, or [3H]-spermine, suggesting that all three polyamines utilize the same transport system. This was further substantiated by uptake kinetic plots which showed that the three polyamines were competitive inhibitors of one another. In terms of receptor specificity, the ranking order among the polyamines was as follows: spermine (apparent K_m, 1.6 μM) > spermidine (apparent K_m, 2.2 μM) > putrescine (apparent K_m, 8.5 μM). This information should prove useful in designing anticancer agents which are intended to utilize this transport system.

INTRODUCTION

It is now well established that proliferating tissues are particularly rich in polyamines and their biosynthetic enzymes (10) and that these molecules are essential for cell proliferation and not merely a consequence of it (for review, see Ref. 12). It is not unexpected, therefore, that an increased exchange of polyamines across the plasma membrane would be associated with cell proliferation. In fact, uptake of polyamines is known to be increased during cell proliferation (9, 16). Pohjanpelto (13) reported that PUT transport is increased in human fibroblasts stimulated to divide and that it is faster in sparsely populated cell cultures. It has also been shown recently (18) that platelet release product and other mitogens stimulated PUT transport and, subsequently, cell growth in cultured arterial smooth muscle cells. Chen and Rinehard (3) correlated the early process of differentiation (and hence cessation of cell proliferation) with decreases in PUT uptake. Overall, these findings suggest that the transport of polyamines into cells is an integral part of the proliferative process.

Polyamine transport into mammalian cells (excluding erythrocytes) is carrier mediated and energy dependent (5, 15) as indicated by the marked negative effect of temperature and metabolic poisons. A similar system has been described also in bacterial cells (8, 19). Like the polyamine-biosynthetic enzymes (12), the transport system seems to be highly regulated, responding rapidly to polyamine antimetabolites such as DFMO (1), an irreversible inhibitor of ornithine decarboxylase, and MGBG (2), a potent inhibitor of S-adenosylmethionine decarboxylase. This adaptive increase in uptake may be similar to that seen with amino acid transport where deprivation results in a derepression of the uptake system (7).

Since polyamine transport is carrier mediated (5, 15), agents which utilize the system, such as the anticancer drug MGBG (4, 5), may be concentrated intracellularly at a gradient 1000-fold greater than the cell exterior so that mw quantities of the drug may be accumulated (11). Because a carrier is involved, it is expected that the system is structurally specific for certain molecular features of the polyamines.

We have recently proposed that utilization of the polyamine transport system might provide a useful strategy in cancer chemotherapy, offering both tissue selectivity and intracellular concentration to the appropriate agent (15). To that end, we have recently demonstrated that the N^1 and N^8 primary amines of SPD are the most critical determinants in transport specificity (15), leaving the N^4 amine for derivatization. Of lesser importance as a determinant is the aliphatic chain length separating the amines. In the present study, we have examined this latter factor in detail using a series of diamine and triamine homologues of PUT and SPD, respectively. The transport specificity is greater for those having chain lengths similar to those of SPD or SPM (but not PUT), even though the latter utilizes the same transport mechanism.

MATERIALS AND METHODS

Chemicals. The abbreviation for PUT homologues having the general structure NH2(CH2)nNH2 is DA_n (for diamine) where n = 3 to 8. The abbreviation for spermidine homologues having the general structure NH2(CH2)_nNH(CH2)_mNH2 is TA_m,n (for triamine) where n = 3 or 4 and m = 3 to 8. The various homologues were obtained as follows: TA_3,4, TA_4,3, and TA_4,4 were synthesized by Neal Stolowich (University of Florida, Gainesville, Fla.) (see Ref. 14); TA_4, n was kindly provided by D. R. Morris (University of Washington, Seattle, Wash.); DA_3, DA_4, DA_5, and DA_6 were obtained from Sigma Chemical Co. (St. Louis, Mo.); and DA_7 was obtained from Tridron/Fukua Chemical Co. (New York, N. Y.).

Cells. Ascites L1210 leukemia cells were maintained by weekly i.p. transplantation in female DBA/2J mice. For uptake studies, 10^6 leukemic cells were inoculated i.p. 4 days prior to use. Cells were collected by peritoneal lavage with RPMI 1640. The cells were washed twice, counted electronically, and adjusted to a density of 5 x 10^6 cells/ml.

Uptake Determinations. The series of diamines and triamines were
studied for their ability to compete with [3H]SPD for uptake into ascites L1210 leukemia cells in vitro. Prewarmed L1210 cell suspensions (5×10^6/ml) were incubated in 1 ml of RPMI 1640 containing 2% 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid-3-(N-morpholino)propanesulfonic acid and 0.2, 0.5, 1.0, 2.0, 5.0, or 10 μM [3H]PUT, [3H]SPD, or [3H]SPM (New England Nuclear, Boston, Mass.) alone or in the presence of 20 or 100 μM polyamine or 100 μM diamine or triamine. The cells were incubated for 20 min at 37°C except for one tube containing 10 μM [3H]PUT, [3H]SPD, or [3H]SPM which was not prewarmed and which was incubated at 0°C to assess non-specific binding. At the end of the incubation, the tubes were centrifuged at 900 x g for 5 min at 0-4°C. The pellet was washed twice with 5 to 7 ml of cold RPMI 1640 containing 1 mM polyamine to displace nonspecifically bound [3H]-polyamine. The pellet was then dried with a cotton swab and dissolved in 200 μl of 1 N NaOH at 60°C for 20 to 60 min. The material was neutralized with 1 N HCl, diluted to 1 ml with distilled water, and transferred to a vial for scintillation counting. Uptake was linear with time from 1 to 40 min and with [3H]SPD concentration up to 30 μM. Results were expressed as pmol [3H]-polyamine taken up per min per mg protein. Uptake data were analyzed for kinetic characteristics with a Hewlett-Packard HP-85 microcomputer programmed for nonlinear regression curve fitting (6).

RESULTS

For all uptake studies, the specific activity of the [3H]-polyamine was adjusted with unlabeled polyamine, so that, even in the presence of a competing triamine or diamine and at the lowest concentration of the [3H]-polyamine, the cpm were at least 300 after subtraction of background radioactivity (20 to 30 cpm). At 20 min, uptake was found to be linear with concentration for all 3 polyamines. Incubations were performed at 0°C as controls for nonspecific binding of [3H]-polyamine and gave cpm that were 6 to 8% of those preparations incubated at 37°C. When labeled cells were washed with buffer containing 0.1 mM cold polyamine, the counts decreased to less than 1% of the preparations incubated at 37°C. The latter was a standard procedure throughout these studies. In all cases, cell washes were performed at 4°C for less than 20 min following cell labeling. Label loss during this period was found by comparable control incubations to be less than 4% of the total cell-associated radioactivity.

Chart 1 shows an uptake kinetic plot of [3H]SPD uptake in the presence and absence of 20 μM PUT or SPM. The slope intersects indicate competitive inhibition, suggesting that all 3 polyamines utilize the same transport mechanism. From the plot and from the apparent Km constants given in Table 1, it is apparent that the carrier specificity is greatest for SPM, then for SPD, and least for PUT. Despite this ranking, real transport as indicated by the apparent V_{max} is clearly greatest for SPD. The chain length specificity of the polyamine transport carrier was assessed by measuring the ability of various diamines and triamines to compete with [3H]PUT, [3H]SPD, or [3H]SPM plus 100 μM diamine. Note that carrier specificity is expressed as the reciprocal of the apparent K_{i} x 10^3, so that positive column height correlates with carrier specificity.

Table 1

<table>
<thead>
<tr>
<th>Polyamine</th>
<th>K_{i} (μM)</th>
<th>V_{max} (pmol/mg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PUT</td>
<td>8.5 ± 0.5</td>
<td>27.3 ± 10.8</td>
</tr>
<tr>
<td>SPD</td>
<td>2.2 ± 0.3</td>
<td>140.5 ± 47.7</td>
</tr>
<tr>
<td>SPM</td>
<td>1.6 ± 0.4</td>
<td>65.8 ± 16.4</td>
</tr>
</tbody>
</table>

* Mean ± S.D. for 4 experiments performed in duplicate.
height correlates positively with the ability of the diamine or triamine to compete with the radiolabeled polyamine and thus with the specificity of the transport system.

For both diamine and triamine homologues, competition was uniformly greatest in the presence of [3H]PUT, then [3H]SPD and [3H]SPM. This is consistent with the apparent $K_m$ constants given in Table 1. Among the diamines, DA$_7$ (with a chain length similar to SPD) showed the greatest competition for polyamine uptake. Competition decreased with chain length so that the shortest diamine, DA$_{3}$, was least effective ($K_m < 500 \mu M$).

In general, the triamines competed much more effectively than the diamines for polyamine uptake (Chart 3; note change in the ordinate scale). In contrast to the competition plot for diamines, that for triamines shows a biphasic profile in transport specificity with the first peak being at $\tau$TA$_3$ rather than at SPD ($\tau$TA$_4$) itself and the second being at $\tau$TA$_4$ and $\tau$TA$_s$, which are similar in chain length to SPM.

The relative uniformity of the effect of the various diamine and triamine homologues on the uptake of all 3 $^3$H-polyamines is apparent in the 2 competition plots (Charts 2 and 3). This would be expected if the same system transported all 3 polyamines.

**DISCUSSION**

In a previous report (15), various N$^1$-N$^6$, or N$^4$-derivatives of SPD were used to determine the specificity of SPD uptake into L1210 cells. The latter was found to be dependent on the availability of the primary amines and to a lesser extent on the aliphatic chain length separating the amines. This information was based on uptake competition studies using N$^4$-benzyl derivatives of $\tau$TA$_3$ (nor-SPD), $\tau$TA$_4$ (SPD), and $\tau$TA$_s$ (homo-SPD). Unexpectedly, it was found that the N$^4$-benzyl derivative of $\tau$TA$_s$ competed much more effectively than that of SPD for [3H]SPD uptake, with the ranking order being $\tau$TA$_s > \tau$TA$_4 > \tau$TA$_3$ (the latter being least effective).

We have now extended this finding further by examining a full complement of diamine and triamine homologues of PUT and SPD, respectively, on the uptake of [3H]PUT, [3H]SPD [previously reported elsewhere (19)], and [3H]SPM. From competition plot profiles of these homologues (Charts 2 and 3), it is apparent that the carrier mechanism has a definite specificity in terms of aliphatic chain length. The greatest specificity is for homologues which are similar in chain length to either SPD (for $\tau$TA$_4$ itself) and the second being at $\tau$TA$_4$ and $\tau$TA$_s$, which are similar in chain length to SPM.

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