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5'-NUCLEOTIDASE ACTIVITY IN CULTURED CELL LINES. EFFECT OF DIFFERENT ASSAY CONDITIONS AND CORRELATION WITH CELL PROLIFERATION

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SUMMARY

The 5'-AMPase activity of the ectoenzyme 5'-nucleotidase has been measured in a variety of cell lines, using intact cells. Human cell types showed two orders of magnitude higher enzyme activity than mouse cell lines. The ectoenzyme is inhibited by adenosine 5'-(α,β-methylene) diphosphate and Concanavalin A. A different extent of 5'-nucleotidase lectin inhibition was observed in the studied cell lines, suggesting that the corresponding ectoenzymes are glycoproteins with a different type or degree, or both, of glycosylation. The 5'-nucleotidase activity increased during subculture and decreased after cell transformation. Generally, the 5'-nucleotidase activity was two- to five-fold higher in monolayer than in suspension cell culture. A relation between cell growth and 5'-AMPase activity was also observed. Enzyme activity increased at the end of the lag phase (glioblastoma cells) or during the exponential phase (the other two cell lines). After confluence, the activity decreased to the initial or even lower range of activity. Observed activity variations with cell proliferation correlate with modifications of 5'-AMPase activity during subculture.

Key words: 5'-nucleotidase; cell proliferation.

INTRODUCTION

5'-Nucleotidase (5'ribonucleotide phosphohydrolase, E.C. 3.1.3.5) catalyzes the hydrolysis of nucleosides 5'-monophosphate to nucleosides and inorganic phosphate. The most abundant cellular 5'-nucleotidase is an ectoenzyme with its active site located at the external face of the plasma membrane. This ectoenzyme is considered a general marker for plasma membranes of eukaryotic cells (9). In addition, the enzyme has been found in lysosomes, in the Golgi apparatus and in cytosolic cell fractions (14,20). 5'-Nucleotidase activity has been detected in many tissues and several cell lines from different species (10,14,18,20,23,26,27).

There are several hypotheses concerning the physiologic role of the intracellular enzyme. Thus, the cytosolic 5'-nucleotidase and AMP desaminase are believed to form a futile cycle between AMP and adenosine (2); the nucleotidase could also be implicated in the removal of IMP excess generated by AMP desaminase (13) or in the production of inosine which is exported from the liver to other tissues (25).

Several physiologic functions have also been suggested for the ectoenzyme (14). The adenosine release occurred in connection with nucleotide scavenging, vasodilation, neurotransmission, hemostasis, and regulation of adenylate cyclase (21). Moreover, an involvement of the enzyme in the control of cell growth, maturation, and differentiation has been proposed. An inverse relationship between 5'-nucleotidase activity and the rate of cell proliferation has been described, suggesting that 5'-nucleotidase could exert a negative control on cell proliferation (23,24). Changes in 5'-nucleotidase activity have been observed during cell maturation in various cell types. Thus, a low 5'-nucleotidase activity reflected an arrest of cells in an early stage of maturation (7). An increase of 5'-nucleotidase activity during cell aging has also been reported (22), whereas a marked reduction appeared in malignant cells when compared to their normal parental cells (18).

The results reported for 5'-nucleotidase activity are however, diverse. Its variability has been described to be a function of species and cell origin (26). A better knowledge of the characteristics of this enzyme in living cells and conditions that modify its activity will contribute to clarify the physiologic role of 5'-nucleotidase. Thus, we have studied the AMPase activity

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in cells from different sources under two assay conditions (monolayer and suspension) at different cell densities as well as the modification of this activity during cell proliferation.

Materials and Methods

Materials. Concanavalin A (Con A), adenosine 5′-(α,β-methylene) diphosphate (AOPCP), $ZnSO_4$, and $Ba(OH)_2$ were from Sigma (München, FRG). [U-^{14}C]adenosine 5′-monophosphate (538 mCi/mmol) was obtained from Amersham (Buckinghamshire, UK), and adenosine 5′-monophosphoric acid from Serva (Heidelberg, FRG). Media, sera, antibiotics and trypsin-EDTA were purchased from Flow Laboratories (Rockville, MD); 24-well tissue culture plates and plastic flasks from Costar (Cambridge, MA).

Cell lines and tissue cultures. Eighteen different cell lines were tested for 5′-nucleotidase activity. The established cell lines were: Eb, Esb, and Esb-M (mouse lymphoma, kindly provided by Dr. V. Schirrmacher, German Cancer Research Center, Heidelberg, FRG); 3T3 and SV-3T3 (mouse fibroblasts); EAC2 (Ehrlich ascitic mouse cells); MG-63 (human osteosarcoma); RD (human rhabdomyosarcoma); HT-1080 (human fibrosarcoma); Rugli (human glioblastoma, a generous gift from Professor B. Odermatt, University Hospital, Zurich, Switzerland); and Patu cells (derived from a human ductal pancreatic adenocarcinoma, a gift of Dr. Mollenhauer, University Erlangen-Nürnberg, Erlangen, FRG). Mb-M and clone MM-14 (mouse skeletal muscle, secondary and clone, myoblasts), and FB-M (mouse fibroblasts) were obtained as described (15). FB-G and FB-HG (human fibroblast cultures derived from gingiva and gingival hyperplasia tissue); BCS-TC1 and BCS-TC2 (cells derived from a human colon adenocarcinoma, obtained from explants in our laboratory).

The following cell lines were cultured in RPMI 1640 medium: Eb, Esb, Esb-M, EAC2, BCS-TC1, and BCS-TC2. The other cell lines were cultured in Dulbecco's modified Eagle's medium. The culture media were supplemented with 10% fetal bovine serum, glutamine (300 μg/ml), penicillin (50 IU/ml), and streptomycin (50 μg/ml). Fetal bovine serum was replaced by 1% chick embryo extract and 20% horse serum for myoblast cell lines, and the medium was supplemented with 10% fetal bovine serum plus 5% horse serum for Patu cells.

Cells were grown to confluence in plastic flasks with 75-cm^2 growth surface area. Cultures were incubated at 37° C in a humidified atmosphere (5% CO_2:95% air, vol/vol). Cells growing on monolayer were harvested at confluence and subcultured by trypsinization (0.05% trypsin, 0.02% EDTA). The medium was changed every 2 d. Cells were tested for mycoplasma contamination by a standard fluorescence staining method based on 4:6-diamidino-2-phenylindole (Bioassay Systems, Woburn, MA).

Enzyme assay. AMPase activity was determined by a radioisotope assay (11). The incubation buffer was 50 m*M* Tris-HCl, pH 7.4, containing 10 m*M* $MgCl_2$ and 0.12 *M* NaCl. For exclusion of nonspecific phosphatases, several controls were assayed in presence of 20 m*M* β-glycerophosphate. No modifications in the AMPase activity were observed in the presence or absence of this reagent, showing that nonspecific acid and alkaline phosphatases were not active at this pH, as also described by other groups (24). Similar results were obtained when the buffer was replaced by cell culture medium. The rate of AMP hydrolysis was measured either in cell suspensions or in monolayers.

The standard assay mixture contained cells, 76 nmole of adenosine 5′-monophosphoric acid and 5×10^{-3} μCi [^{14}C]AMP (538 mCi/mmol) in a total volume of 500 μl. The assay mixture was incubated at 37° C in a shaker for 15 min. The reaction was stopped by adding 0.1 ml of 0.3 *M* $ZnSO_4$ followed by 0.1 ml of 0.3 *M* $Ba(OH)_2$ as indicated by Sun et al. (24). Nonhydrolyzed [^{14}C]AMP was sedimented at 12 000 rpm for 15 min in an Eppendorf 5414 centrifuge. An aliquot of the supernatant was measured in a scintillation counter (Beckman LS 3801). Duplicates were routinely performed. Blanks without cells were considered to evaluate nonenzymatic hydrolysis. 5′-Nucleotidase activity was expressed as nanomoles of substrate hydrolyzed per cell for 15 min. Under the assay conditions, the hydrolysis of 5′-AMP by intact cells was found to be linear for at least 30 min of incubation at 37° C.

Intracellular activity was determined by subtracting the activity of the intact cells, from the total activity (disrupted cells). Whole cell homogenates were prepared, with an appropriate number of cells, by sonication in the incubation buffer at 4° C. The standard assay was performed after lysis.

In the inhibition assays, adenosine 5′-(α,β-methylene) diphosphate (AOPCP) and concanavalin A (Con A) were also added to the incubation mixture.

Protein concentration was determined according to Bradford (3).

Cell suspension assay. Cells growing in suspension were sedimented by centrifugation (1,500 rpm, 5 min) and the cell pellet, after washing with phosphate buffered saline (PBS), was resuspended in the incubation buffer.

Cells growing in monolayer were removed from the culture flasks by trypsinization, and the cell suspension was centrifuged at 1,500 rpm for 5 min. Cells were resuspended in supplemented culture medium and maintained in suspension for 1 h at 37° C. After centrifugation, the supernatant was removed, and the cells washed 3 times with PBS and resuspended in the appropriate volume of incubation buffer; 5′-nucleotidase activity and protein concentration were determined.

Monolayer assay. Different numbers of cells, obtained as described in the cell suspension assay, were seeded in multiwell culture dishes. The monolayers were formed under standard culture conditions. After 2 to 3 d of culture the medium was removed and the cells were washed with PBS. Several wells were trypsinized to evaluate the actual cell number used in each assay; the other were used immediately for the 5′-nucleotidase assay. The monolayers were covered with 500 μl of assay mixture and used for 5′-nucleotidase activity assay.

5′-Nucleotidase activity during cell adhesion and spreading was measured at different time intervals

after seeding the appropriate number of cells in 24-well tissue culture plates, in 400 μl of culture medium. The incubation was initiated by addition of 100 μl of substrate solution without removing the culture medium. The number of cells attached and the degree of spreading were determined in parallel cultures.

Growth curves. Confluent cultures were trypsinized, and the cells were then sedimented and resuspended in growth medium; 3.5×10^3 cells/cm² for FB-HG and Rugli and 3×10^4 BCS-TC2 cells/cm² were seeded in Falcon plastic flasks (25 cm²). The culture medium was changed every 2 d. Every day cell monolayers were washed with PBS and removed by trypsinization. The cell number was determined in a cell counter (Coulter Electronics, Luton, England). The enzyme activity of intact and disrupted cells was obtained at each time as a function of cell number. The value of 5'-nucleotidase activity per cell was always determined at cell densities in which the enzyme activity vs. cell number was linear.

Results and Discussion

5'-Nucleotidase activity in different cell lines. The values of 5'-nucleotidase activity described for different cell lines are very heterogeneous and, in several cases, contradictory. We have analyzed a great number of cell lines propagated in culture to establish standard assay conditions to avoid this heterogeneity in further studies. The enzyme activity was determined in several experiments for each cell type. The results are shown in Table 1. To prevent contamination by cytosolic enzyme, the activity was measured using intact cells (either growing in monolayer or in suspension culture, and cells freshly harvested from the monolayer). No contamination by nonspecific phosphatases was observed.

Mouse and human cells of different origin were tested. The mouse cell lines showed a low activity of 5'-nucleotidase, in agreement with Trams and Lauter (26). In human cell lines the activity was considerably higher than in the mouse cell lines; however, pronounced differences in the 5'-nucleotidase activity between different human cell types were observed (Table 1). In the monolayer assay, the highest activity (934×10^{-5} nmole of 5'-AMP hydrolyzed per cell) was obtained for gingival fibroblast cells and the lowest for adenocarcinoma-derived cell lines (36×10^{-5} nmole of 5'-AMP hydrolyzed per cell); HT-1080 cells did not present any detectable 5'-AMPase activity.

Raz et al. (18) suggested that the variation in the enzyme activity between different cell types may be due to differences in cell size or number of active enzyme units per cell. As indicated above, the activity of 5'-

TABLE 1

ACTIVITY OF 5'-NUCLEOTIDASE IN MOUSE AND HUMAN CELL LINES

Cell Line	Origin	Type of Growth	Activity[a] × 10^5	
			Suspension[b]	Monolayer
Mouse				
Eb	T-cell lymphoma	suspension	0.46 ± 0.19	—
ESb	T-cell lymphoma	suspension	0.41 ± 0.13	—
ESb-M	T-cell lymphoma	monolayer	n.d.	0.29 ± 0.05
Mb	myoblast	monolayer	0.26 ± 0.13	1.12 ± 0.39
MM-14	myoblast	monolayer	0.17 ± 0.12	0.51 ± 0.34
EAC2	ascitic	monolayer	n.d.	1.62 ± 1.12
3T3	fibroblast	monolayer	0.05 ± 0.03	0.15 ± 0.05
SV-3T3	transformed fibroblast	monolayer	no activity	no activity
FB-M	primary fibroblast	monolayer	0.18 ± 0.09	1.09 ± 0.15
Human				
FB-G	gingival fibroblasts	monolayer	610.3 ± 55.3	934.5 ± 56.7
FB-HG	gingival hyperplasia fibroblasts			
	primary culture	monolayer	140.2 ± 48.9	360.1 ± 53.2
	Passage 15	monolayer	340.6 ± 60.4	632.3 ± 35.4
Rugli	glioblastoma	monolayer	38.7 ± 9.6	115.3 ± 24.0
MG-63	osteosarcoma	monolayer	30.5 ± 7.2	61.4 ± 8.4
RD	rhabdomyosarcoma	monolayer	27.1 ± 3.5	67.0 ± 13.2
Patu	ductal pancreatic adenocarcinoma	monolayer	26.1 ± 8.9	79.7 ± 27.3
BCS-TC1	colon adenocarcinoma	monolayer	5.5 ± 2.3	35.6 ± 10.2
BCS-TC2	colon adenocarcinoma	multilayer	8.7 ± 5.7	36.7 ± 10.0
HT-1080	fibrosarcoma	monolayer	no activity	no activity

[a] AMPase activity is expressed as nanomoles of AMP hydrolyzed per cell in 15 min. In all cases the enzyme activity was determined at cell densities where the rate of AMP hydrolysis increased linear with the cell number. Values are the mean ± SD of five or more experiments with triplicate measurements in each experimental group, n.d. = not determined.

[b] Cells growing on monolayer were freshly harvested and the activity of 5' nucleotidase was determined in the cell suspension as described in Methods.

nucleotidase in intact cells is expressed as nanomoles of AMP hydrolyzed per cell in 15 min; but these values can also be related to total cellular protein to avoid the potential influence of cell size on the obtained activity. The differences in the 5'-nucleotidase activity were maintained when the human cell lines were considered. For example, the activities of FB-HG, Rugli, and BCS-TC2 cells in suspension were 3084, 1220, and 170 nmole 5'-AMP hydrolyzed in 15 min/mg of cell protein, respectively. Therefore, the first possibility can be ruled out, and the differences in the activity very likely represent a different number of active enzyme units.

5'-Nucleotidase activity in transformed human cell lines was low or even undetectable in some cases, as for HT-1080 cells. This has previously been related to spontaneous or induced cell transformation and to the "immortal" features of some cultured cells. It reflects a reduced activity of the catabolic pathway of 5'-nucleotides and thus an enhanced pool of nucleotides for recycling in the anabolic process. This defect in the catabolic pathway of 5'-nucleotides in neoplastic cells may therefore play a role in their unlimited proliferation ability (18,23,24).

Culture conditions that affect 5'-nucleotidase activity. The 5'-nucleotidase activity was two- to fivefold higher in cells assayed in monolayer than in the same cells assayed in suspension (freshly harvested cells) (Table 1). This could result from the trypsin treatment by damage to plasma membrane-bound enzymes. But this possibility was ruled out by comparing the enzyme activity of freshly harvested cells with that of cells maintained in suspension for 30, 60, 120, and 240 min after trypsinization, in complete culture. After this time in culture, possible damage of cell surface proteins should be reverted, but no increase in 5'-nucleotidase activity per cell was observed. DePierre and Karnovsky (4) also showed that trypsin treatment had no effect on the 5'-nucleotidase enzyme of intact cells, and Baron et al. (1) indicated that the enzyme molecule is protected against the proteolysis.

The enhancement of enzyme activity in the monolayer assay could be due to a modification in the number of active sites exposed, as well as to a better access of the substrate to the active sites when the cell is spread. This possibility was investigated. We have performed cell adhesion and spreading studies, but no modification in the activity of the ectoenzyme was detected during the whole cell attachment process in the three lines considered. In this way, our results indicate that the change in cell shape neither increases the accessibility of the substrate nor the number of active sites exposed.

Stanley et al. (20,21) have reported a movement of the enzyme between the cell surface and intracellular vesicles, independent of enzyme synthesis and degradation. The intracellular 5'-AMPase accounted only for 25, 21, and 8% of the total activity of FB-HG, Rugli, and BCS-TC2 cells, respectively (data not shown; *see* also Fig. 3). Thus, internalization of the cell surface 5'-AMPase or migration from an intracellular location to the cell surface would not explain the enzyme activity differences observed between suspension and monolayer assays.

A relationship between the cell density and the AMP hydrolysis was also observed. The rates of 5'-AMP hydrolysis as a function of the cell number are shown in Fig. 1. In the suspension assay, the rate linearly increased with the cell density up to 4×10^4, 12×10^4, and 25×10^4 cells per assay for FB-HG, Rugli, and BCS-TC2 cells, respectively, and leveled off thereafter (Fig. 1). In the monolayer assay the linear interval ended at 2×10^4, 4×10^4, and 20×10^4 cells per assay for FB-HG, Rugli, and BCS-TC2 cells, respectively. A stabilization of AMP-hydrolysis rate was obtained at 20 to 25 nmole hydrolyzed in 15 min in BCS-TC2 cells when the number of cells was higher than 40×10^4 (suspension assay; similar results were obtained in the monolayer assay). Inasmuch as the substrate is still available and v_0 (initial rates) values were always considered, this loss of linearity and further stabilization must be due to cell density effects. We have obtained similar results in other cell lines showing low 5'-AMPase activity: RD, Patu, and mouse T-cell lymphoma. Thus the activity dependence with the cell number could explain the

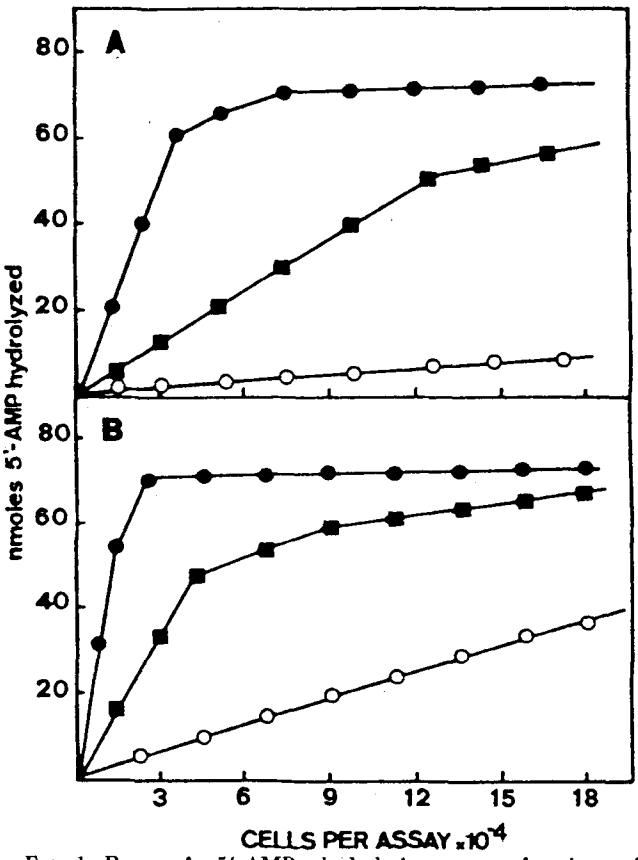

FIG. 1. Rate of 5'-AMP hydrolysis as a function of cell density. 5'-AMP hydrolysis as a function of the density of primary FB-HG (●—●), Rugli (■—■), and BCS-TC2 (○—○) cells are shown. The assay was performed in 0.5-ml reaction volume, at 37° C for 15 min, with (*A*) freshly harvested cells from confluent cultures and (*B*) cells growing in monolayer, 2 d after seeding, prepared as described in Materials and Methods. The values are the mean of eight experiments with four measurements in each experimental group.

differences described by some authors for certain cell lines because, although the activity is expressed in the same units, the assays are performed with a different cell number. Therefore, a correct determination of 5'-nucleotidase activity requires measurements at a different number of cells per assay.

Nakamura et al. (17) have reported various enzyme activities related to cell growth which are reciprocally regulated by cell density via direct cell-to-cell contacts. These contacts are known to be modulated by cell surface glycosylation (12). Our results suggested that 5'-nucleotidase is also modulated by cell density. The different degree of the cell density effects on the AMPase activity of the studied cell lines could be related to a different cell surface glycosylation. Thus it would be interesting to analyze the effect of lectins, such as Con A, in the 5'-nucleotidase activity of such cell lines.

Inhibition assays. The AMPase activity of the 5'-nucleotidase was inhibited by the lectin Con A (0.5 μM) indicating that the enzyme is a glycoprotein (Fig. 2). In the monolayer assays of FB-HG and Rugli cells 85% inhibition was obtained whereas only 60% was detected for BCS-TC2 cells. The inhibition by Con A was higher in the suspension assay; values of 90, 95, and 75% inhibition were obtained for FB-HG, Rugli, and BCS-TC2 cells, respectively. This difference on the inhibition values might be due to a different type or degree of glycosylation of the enzyme in the studied cell lines, which could explain the changes in the 5'-AMPase activity for BCS-TC2 cells at high cell density.

In view of other enzymes showing AMPase activity, control studies on intact cells were performed using specific inhibitors. The ecto 5'-nucleotidase activity from both monolayer cultures and suspended cells was inhibited by AOPCP (25 μM). Activity values represent 3 to 8% of the control samples (Fig. 2). This inhibition was total when the concentration of AOPCP was slightly increased. This total inhibition strongly supports the notion that the determined enzyme activity only reflects 5'-nucleotidase activity (8) and confirms the absence of nonspecific phosphatases.

Effect of subculturing and cell growth phase on 5'-nucleotidase activity. The 5'-nucleotidase activity increased during the subculture of FB-HG cells from 360×10^{-5} nmole of AMP hydrolyzed per cell for primary cells to 632×10^{-5} nmole of AMP hydrolyzed per cell in Passage 15. Similar results were obtained with colon adenocarcinoma cells: activity increased from 8.7×10^{-5} to 14.2×10^{-5} and 19.1×10^{-5} nmole of AMP hydrolyzed per cell in Passages 30, 60, and 90. It was also observed in mouse Ehrlich ascitic (EAC2) cells. Primary EAC2 cells completely lacked the enzyme activity, but values of 1.62×10^{-5} nmole of 5'-AMP hydrolyzed per cell were obtained after Passage 4, showing a gradual increase during subcultivation. This increase in 5'-nucleotidase activity has also been observed in cells during subculture when compared with cells growing out from explants and with cells freshly isolated from tissues (10,22,23). However, the activity of some cell lines remained constant through different passage levels, as occurred with Rugli cells.

Sun et al. (23) have related the increase in 5'-nucleotidase activity during subculture with the increase of this activity during cell exponential growth phase. However, Lelièvre et al. (16) indicated that specific 5'-AMPase activity decreased in membrane preparations from cells in the stationary phase compared with actively growing cells. In view of this apparent contradiction, we performed studies throughout the whole proliferation curves. We also tried to explain the different behavior of 5'-nucleotidase activity with subculture in three human cell lines: FB-HG, BCS-TC2, and Rugli. To avoid possible cell density effects (*see* above), assays were performed as described in Materials and Methods.

Figure 3 shows the enzyme activity of three human cell types at different times after seeding. The length of the lag phase at the seeding density used was 1 d for FB-HG and Rugli cells. Rugli cells exhibited a clear exponential growth phase with a doubling time of 12 h; FB-HG cells were growing with a doubling time of 42 h. The growth curve of BCS-TC2 cells showed a lag phase of 2 to 3 d and a doubling time of 38 h.

The 5'-nucleotidase activities changed during the growth curve in a characteristic manner for each cell line (Fig. 3). During the lag phase, the activity of FB-HG remained constant. The maximum activity was obtained during the logarithmic phase at 6 to 7 d culture with a

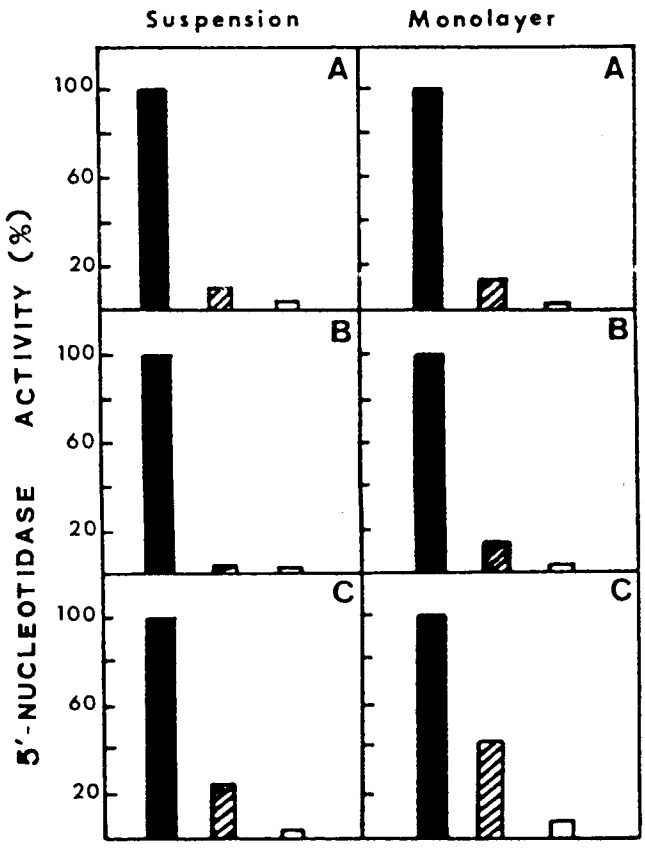

FIG. 2. Effect of Con A (0.5 μM, ▨) and AOPCP (25 μM, □) on 5'-nucleotidase activity of FB-HG (*A*), Rugli (*B*), and BCS-TC2 (*C*) cells. Inhibition assay was performed as described in Materials and Methods with freshly harvested cells from the monolayer (*left*) and with cells growing on monolayer (*right*). All data are expressed as percentage of control values (■).

gradual reduction after 8 d of culture. At confluence the 5'-nucleotidase activity reached a higher value than at the beginning; however, when the culture was maintained at confluence, the activity decreased.

In Rugli cell cultures a fast rise on the enzyme activity was observed during the lag phase, followed by a decrease between Day 2 or 3 of culture. At confluence, the 5'-nucleotidase activity decreased to the initial levels. The activity of BCS-TC2 cells increased gradually during the logarithmic phase but further on it decreased continuously. The maximum activity was observed at about 8 to 9 d of culture. At high cell density saturation the activity declined to a 77% of the initial activity.

The intracellular 5'-nucleotidase activity, determined after cell lysis, exhibited the same time course as the membrane-associated enzyme (Fig. 3). During cell proliferation, both ectoenzyme and intracellular 5'-nucleotidase activities increased but their ratio remained almost constant. Therefore, a

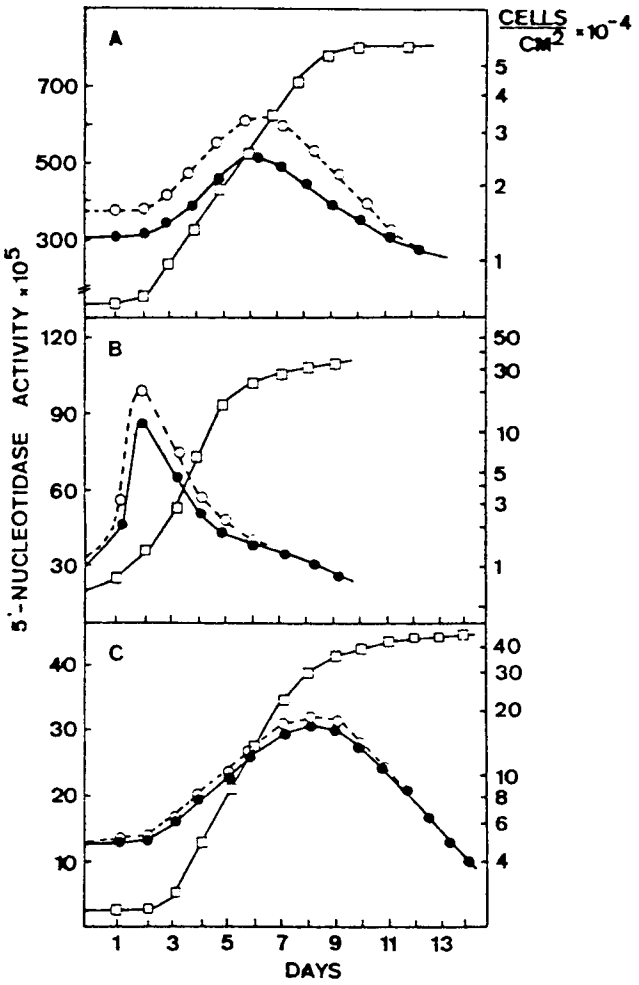

FIG. 3. Growth curves and 5'-nucleotidase activity of (*A*) FB-HG (Passage 15), (*B*) Rugli, and (*C*) BCS-TC2 cell lines. Every day the cells were removed from the monolayer by trypsinization. Cell number (-□—□) and 5'-nucleotidase activity of intact cells (●—●, ectoenzyme activity) and disrupted cells (O—O, total activity) were determined. 5'-Nucleotidase activity is expressed as nanomoles of 5'-AMP hydrolyzed per cell in 15 min ($\times 10^5$). All data represent mean values of three determinations with duplicate values.

dynamic relationship between the two pools of enzyme could be suggested, as described (20,21).

The increase observed in the 5'-AMPase activity of Rugli cells after 2 d of culture correlates with the increase found in the monolayer assay when compared with the suspension assay. After this time in culture the 5'-nucleotidase activity sharply declines to the initial level. No fluctuations on the enzyme activity were observed with progressive subculture. This suggests a role for 5'-nucleotidase on the cell proliferation of Rugli cells. Thus the increase of 5'-AMPase activity, by mechanisms that imply either activation or synthesis of the enzyme, is associated with requirements of nucleotides for the synthesis of nucleic acids.

The variation of 5'-AMPase activity during the growth curves of FB-HG and BCS-TC2 is different from that of the Rugli cells. This could be related to a different proliferation rate, indicating that cells with slower growth rate could reach their maximum 5'-nucleotidase activity later than cells with a faster one. It could therefore explain the increase observed in their activity during the subculture, because the cells were harvested on Day 7 after seeding. However, the differences between monolayer and suspension assays must be explained by other reasons, such as loss of enzyme molecules by shedding off of plasma membrane vesicles during the detachment process (18, 28); inactivation of the enzyme; loss of active sites due to a modification in the membrane structure with or without alteration of the lipid environment (14,19); and the presence of activity-modifying factors (14,29). Furthermore, recent reports indicate that the enzyme in vitro interacts with filamentous actin (6) and with certain components of the extracellular matrix (5), modifying the 5'-nucleotidase activity.

In summary, we can conclude that: a) 5'-Nucleotidase in cell lines with low specific activity is density dependent and is probably related with the type or degree of glycosylation. b) Differences between monolayer and suspension assays suggest the importance of environmental factors on this activity. c) Activity changes during cell proliferation were observed showing a different pattern for each cell type. These changes could explain the maintenance or variations of 5'-nucleotidase activity during subculture. d) Cells that present constant 5'-AMPase activity throughout passage levels seem to have a direct relationship between the enhancement of enzyme activity and nucleotide requirements. However, 5'-nucleotidase changes with cell proliferation in cells showing changes during subculture seem to be dependent not only on nucleotide requirements but also could reflect continuous adaptation to culture conditions.

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