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ENZYME ACTIVITIES IN MEMBRANES FROM THREE PHENOTYPES OF THE MURINE PLASMOCYTOMA MOPC 173, CULTIVATED *IN VITRO*

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SUMMARY

Cell surface and endoplasmic reticulum membranes were isolated from mouse plasmocytoma cells in culture. The distributions of membrane-bound enzyme activities over sucrose gradient fractions differed for epithelioid and fibroblastic cells.

It is shown that microsomal enzymes are present in plasma membranes when isolated from contact-inhibition sensitive cells. When epithelioid cells reach confluence, a reduction in the enzyme activities of the plasma membrane fractions was found.

INTRODUCTION

It has been shown that certain enzyme activities present in the plasma membrane[1–3] or the endoplasmic reticulum[4–6] may arise or become modified during cellular differentiation. We are investigating the possibility that certain "marker" enzymes, by their presence and localization, may be characteristic of a particular state of differentation.

In a previous communication[7] we described the isolation of two lines of differentiated cells from the murine plasmocytoma MOPC 173, A third type has been isolated by Dr Samaille of the Institut Pasteur, Lille (France). These cell lines seemed appropriate for an investigation of the distribution of membrane-bound enzymes. Previously[8] we had identified plasma membranes and endoplasmic reticulum enzymes for the fibroblastic cell type MF2. In this communication we shall compare these enzymes in two other phenotypes and show that the concentration of serum in the culture medium may affect the localisation of enzyme activities in reticular membranes and that in cells which exhibit contact inhibition these enzyme activities undergo modification at confluence. This phenomenon is not found at similar population densities in cell types which are insensitive to contact inhibition.

MATERIAL AND METHODS

Cell lines

Analyses were carried out on two cell lines derived from the myeloma MOPC 173, namely ME2 and MF2, both grown on plastic or glass. The characteristics of

MF2 cells have already been described[7,8]. ME2 cells stop growing when the cells come into contact with each other[3]. Transplanted into a mouse, these epithelioid cells do not give rise to tumours. The culture conditions were identical except that the concentration of calf serum was 10% for the MF2 cells and 2% for the ME2 line.

MF2 S cells were provided by the "Institut Pasteur" at Lille. These are MF2 cells adapted to grow in suspension culture in a medium containing horse serum.

Isolation of plasma membranes

The technique for the isolation of plasma membranes originally developed for MF2 cells was found to be satisfactory for both cell lines[8].

Enzymatic assays

With all cell types the same enzymic tests were used as described previously for MF2 cells[8].

RESULTS

The results with ME2 cells are presented schematically in Table I, and those obtained with MF2 S in Table II.

In reporting the results of enzyme analysis, we have used the same definitions of recovery and purification as given previously[8].

The kinetics of the enzyme reactions were linear with respect to time.

Distribution and recovery of proteins

With a given cell type the distribution and recovery of proteins from all gradients were comparable to those obtained previously with MF2 cells[8].

Enzyme activities

Plasma membranes

The activity of $(K^+ + Na^+)$-stimulated Mg^{2+}-ATPase was essentially the same in MF2 S, ME2 and MF2 cells[8]. 5'-Nucleotidase of ME2 cells was found mainly in Bands A_1 and B_1 and A_2' and A_2 of a second gradient (Table I). It should be noted, however, that 2% of the initial activity was found on Bands B_2 and C_2 of this gradient. This apparently broad distribution in the gradient (Tables I and II) depends on the conditions of assay. The activating effect (50%) of Mg^{2+} on AMP hydrolysis was only found in fractions containing $(K^+ + Na^+)$-stimulated Mg^{2+}-ATPase. Although its specific activity is lower in ME2 and MF2 S (4–4.6 μmoles P_i/h per mg) than in MF2 (9-10 μmoles P_i/h per mg), the characteristics of this enzyme in the two cell types corresponded to those of the enzyme in MF2 cells. Thus it would appear that 5'-nucleotidase activity is specific for plasma membranes of the three cell lines.

The $(K^+ + Mg^{2+})$-stimulated *p*-nitrophenylphosphatase occurred in larger amounts in D_1 and P_2 fractions than in any other fraction. However, its specific activity is much higher in the B_1 fraction which corresponds to the plasma membrane fraction than in D_1 and P_2. This type of observation will be described for other enzymes and we shall discuss this very important point in the final part of this paper.

Endoplasmic reticulum enzymes

UDPase activity in fibroblasts grown in suspension culture was similar to

TABLE I

ME2 CELLS: SPECIFIC ACTIVITIES OF ENZYMES IN THE CRUDE FRACTIONS AND IN THE DIFFERENT BANDS OF SUCROSE GRADIENTS

Abbreviations: L, lysate; P, pellet; S, supernatant 27000 × *g*. L.D., limit of detection. (a) Preparation consisting of a single gradient. (b) Preparation consisting of two gradients.

Enzyme		*Crude fractions*			*Bands of the gradients–nomenclature–density*					
		L	*S*	P_1	A'_2 *1.12/1.14*	A_1 A_2 *1.14/1.16*	B_1 B_2 *1.16/1.18*	C_1 C_2 *1.18/1.20*	D_1 D_2 *1.20/1.22*	P_2 *1.22*
$(K^+ + Na^+)$-stimulated Mg^{2+}-ATPase*	(a)	0.9	0	1.3		4	2	0	0	0
	(b)	1.5	0	1.8	3	12.6	0	0	0	0
5′ Nucleotidase* + Mg^{2+}	(b)	1.1	0.3	1.3	4.6	5	0.5	0.3	L.D.	L.D.
− Mg^{2+}	(b)	1.1	0.3	1.2	3	3	0.5	0.3	L.D.	L.D.
$(K^+ + Mg^{2+})$-stimulated *p*-nitrophenylphosphatase**	(a)	3.3	1.6	4		4	3.7	0.8	0.6	1.8
UDPase*	(b)	5.3	2	6.6	0	0	0	1.3	6.7	0
Alkaline phosphatase*	(a)	0.2	0.14	0.2		0.6	0.6	0	0	0.1
	(b)	0.1	0.1	0.1	0.2	0.3	0.1	L.D.	0.1	L.D.
NADH: cytochrome *c* reductase***	(a)	0.15	0.01	0.2		0.05	0.06	0.03	0.04	0
Glucose-6-phosphatase*	(a)	0.3	0.2	0.4		1.2	1.1	1.2	0	0.7
	(b)	0.3	0.2	0.4	2.4	2.4	0.9	0.4	0	1
Acid phosphatase*	(a)	1.9	0	1.9		3.6	2.1	1.1	0.7	1.1

* Specific activities expressed as μmoles P_i/h per mg of protein.
** Specific activities expressed as μmoles *p*-nitrophenol/h per mg of protein.
*** Specific activities expressed as μmoles cytochrome *c* reduced/h per mg of protein.

TABLE II

MF2 S CELLS: SPECIFIC ACTIVITIES OF ENZYMES IN THE CRUDE FRACTIONS AND IN THE DIFFERENT BANDS OF SUCROSE GRADIENTS

We have not separated the fractions from MF2 S cells on two successive gradients, since the lability of the enzyme activities did not permit this. Abbreviations: L, lysate; P, pellet; S, supernatant 27000 × *g*. L.D., limit of detection.

Enzyme	*Crude fractions*			*Bands of the gradients–nomenclature–density*				
	L	*S*	P_1	A'_1 *1.14/1.16*	B_1 *1.16/1.18*	C_1 *1.18/1.20*	D_1 *1.20/1.22*	P_2 *1.22*
$(K^+ + Na^+)$-stimulated Mg^{2+}-ATPase*	0.6	0	0.6	8.7	3.4	0	0	0
5 Nucleotidase* $+ Mg^{2+}$	0.4	0	0.4	4	3.5	4.5	1.5	0
$- Mg^{2+}$	0.4	0	0.4	2.6	2.5	4.5	1.5	0
$(K^+ + Mg^{2+})$-stimulated *p*-nitrophenylphosphatase**	0.3	0.3	0.3	0.5	1.9	0	0.22	0.3
UDPase*	9	9.3	8.9	20.6	20	16	18	14
Alkaline phosphatase*	0.4	0.5	0.4	0	0.66	0.7	2.2	0.8
NADH: cytochrome *c* reductase***	0.08	0	0.08	0.28	0.09	0.04	0.03	0
Glucose-6-phosphatase*	0.3	0	0.3	0.5	0.75	0.4	0	0
Acid phosphatase*	0.2	0	0.2	1.8	L.D.	0.6	0.2	0

* Specific activities expressed as μmoles P_i/h per mg of protein.
** Specific activities expressed as μmoles *p*-nitrophenol/h per mg of protein.
*** Specific activities expressed as μmoles cytochrome *c* reduced/h per mg of protein.

that in monolayers except that the specific activity of the enzyme was lower (9–20 instead of 600 μmoles P_i/h per mg).

The localization, degree of purification, and recovery of alkaline phosphatase were indistinguishable in the two types of fibroblasts.

UDPase activity in ME2 cells was largely solubilized and present in the supernatant fraction (85%), but a residual activity was present at interfaces 1.18/1.20 and 1.20/1.22.

The activity of alkaline phosphatase in ME2 cells was found in Fractions A_1, B_1 (15%) and P_2 (20%) of the first gradient. Its specific activity was much higher in the light region of the gradient. If A_1 and B_1 were pooled and run on a second gradient the resultant purification remained the same and alkaline phosphatase activity was recovered in bands A_2' and A_2 (interfaces 1.12/1.14 and 1.14/1.16, respectively) (Table I). In epithelial cells only 35% of its original concentration in the lysate was recovered.

When ME2 or MF2 S cells were fractionated, 92–98% of the initial activity of NADH: cytochrome *c* reductase is lost, due to solubilization or inactivation. The specific activity of this enzyme is higher in the lysate than in the gradient layers.

Similarly, in fibroblasts grown in suspension, glucose-6-phosphatase was almost completely solubilized and therefore excluded as a marker. On the other hand, for epithelioid lines, 60% of the total activity of this enzyme was found in the nuclei, 20% in the plasma membranes and 10% in the $27000 \times g$ supernatant. Its specific activity in Fraction P_2 represented a purification of 2-to 3-fold that of the lysate. While the enzyme may be located around the nucleus, the degree of purification varied from 4- to 8-fold in the plasma membrane fractions.

Acid phosphatase was localized in the plasma membranes (8%) and in the nuclei (27%) in ME2 cells. The recovery of activity was only about 35% of the initial total activity. The degree of purification in each fraction was low. The 22% of total activity recovered on a gradient of MF2 S cells was similarly divided between Fractions C_1 and A_1. In the latter fraction a 9-fold purification was reached. It would appear that whereas in MF2 cells this enzyme activity is a marker for lysozomes and is absent from plasma membranes, in MF2 S and ME2 cells it is found in small quantities with a high degree of purification.

The results obtained for all the markers in each cell line are summarized in Table III. It is assumed that the activities are not solubilized.

The effects of serum

The differences which have been found between ME2, MF2 and MF2 S cells might be attributed to the different concentrations of serum in the culture media. We were especially interested in the cell lines ME2 and MF2 which grow in 2 and 10% calf serum, respectively. When ME2 cells were grown in 10% serum the specific activities, distributions, recoveries and degrees of purification of markers were identical to those described. Correspondingly, analyses carried out on MF2 cells grown in 2% serum were identical to those in 10%. Nevertheless the concentration of serum does have some effect on the cells, since growth of these epithelioid cells ceases at higher cell densities in 10% serum than in 2%[3].

The effects of cell density

The results for the ME2 cell line presented here were obtained with growing cells (40000 cells/cm^2). At densities of the order of 80000 cells/cm^2 we have found

TABLE III

ENZYME ACTIVITIES AS MEMBRANE MARKERS

Type of membrane	*Enzyme*	*Cell lines*	
		MF2 S	*ME2*
Plasma	$(K^+ + Na^+)$-stimulated Mg^{2+}-ATPase	+	+
	5'-Nucleotidase	+	+
Reticular	UDPase	+	−
	Glucose-6-phosphatase	−	+
	Alkaline phosphatase	+	±
Endoplasmic	NADH: cytochrome *c* reductase	−	−
Lysosomes	Acid phosphatase	−	±

that there is a significant reduction in the enzyme activities of the plasma membrane fractions. Specific activities of the 5'-nucleotidase and of the transport ATPase were 0.3 μmoles P_i/h per mg and 0.1 μmoles P_i/h per mg, respectively instead of 3.7 and 12.6 for these two markers in non-confluent cultures (Table IV). In contrast, the activities which characterize the reticular fractions did not alter at confluence. A similar phenomenon was observed when ME2 cells reached confluence in 10% serum.

The activities of marker enzymes for plasma membranes in MF2 cells were similar no matter what the cell density (50000–200000 cells/cm^2). The only reduction in enzyme activities in these cells was observed when the multicellular layer become detached from its support (glass or plastic).

TABLE IV

SPECIFIC ACTIVITIES OF MARKERS FOR PLASMA MEMBRANES IN GROWING AND CONTACT INHIBITED CELLS

The activities are expressed as μmoles of phosphate liberated per h per mg of protein.

	5'-Nucleotidase	*$(K^+ + Na^+)$-stimulated Mg^{2+}-ATPase*
Growing cells	3.7	12.6
Contact inhibited cells	0.30	0.1

DISCUSSION

We have attempted to establish whether enzymes studied in the three phenotypic variants of the murine plasmocytoma MOPC 173 have the same characteristics of localization, specific activity, and lability as in other cell systems.

Each enzyme activity has been determined under defined conditions, but in no case we have attempted to define the K_m values. Despite this we think that we are

measuring the same activity in the three cell types for the following reasons. It is well established that the three phenotypes have the same origin[9] and studies on other systems show that the conditions of pH which we have used in the assays are optimal.

In all types of cell studied we have found that the surface membranes are well localized and their specific enzymes have been purified up to 8-fold. The residual activity of these enzymes never amounted to more than 30% of the total initial activity, no matter what the cell line, marker enzyme, or number of gradients used in the preparation. We have attempted to explain this phenomenon in a previous communication[8]. The $(K^+ + Na^+)$-stimulated Mg^{2+}-ATPase and 5′-nucleotidase appear to be satisfactory markers for plasma membranes in the three cell types. However one question is how to explain the difference in 5′-nucleotidase activities in the presence and absence of Mg^{2+}. Either the 5′-nucleotidase has an absolute requirement for Mg^{2+} and its activity is superimposed on a non-specific monophosphatase, or the 5′-nucleotidase is activated by Mg^{2+} (here by 50%), or this marker corresponds to two activities: one not activated by Mg^{2+}, present in the three lightest fractions and characteristic of the reticulum, the other in A_1 and B_1 and subject to activation. It is possible that this first type of activity reflects the presence of a reticular phosphatase on plasma membranes. The fact that the activating effect of Mg^{2+} is not seen in Fraction P_1 indicates the extent of purification of the 5′-nucleotidase in plasma membranes. It is unlikely that the hydrolysis of AMP in the absence of Mg^{2+} is due to the monophosphatase activity of glucose-6-phosphatase and alkaline phosphatase, since the three activities do not correspond and the degrees of purification in a given fraction are different.

Although UDPase and alkaline phosphatase are present in plasma membranes, these activities may characterize endoplasmic reticulum membranes in fibroblast type cells, and to a lesser extent in ME2 cells. Glucose-6-phosphatase is a satisfactory marker for the epithelioid line. NADH: cytochrome *c* reductase is only a good marker for MF2 cells[8]. It is interesting that on these criteria the MF2 S cell line is much more similar to MF2 than ME2, despite the different culture conditions of the fibroblasts.

We have tried to identify membrane markers according to two criteria: specific enzyme activity and % recovery.

The term "marker" does, however, not necessarily mean that the enzyme under study should be exclusively present in one organelle. For instance, in the case of the ME2 cells, the specific activity of the glucose-6-phosphatase is higher in Fractions A_1 and B_1 or A'_2 and A_2 than that in any other fraction (although most of the total activity (60%) resides in the nuclei); this is highly significant and could allow the use of the term "plasma membrane marker"; it has been found that liver plasma membrane could contain the glucose-6-phosphatase[10]. However, the other interpretation would be that the glucose-6-phosphatase is a true endoplasmic reticulum membrane marker, the ME2 plasma membranes being largely contaminated by endoplasmic reticulum.

Using these marker enzymes it is possible to estimate the extent of reticular contamination of the plasma membranes. Contamination, defined as before[8], ranged from 1–6% for fibroblast type cells, compared with 15–20% for the epithelioid type (Table V). In ME2 cells alkaline phosphatase and glucose-6-phosphatase activities are localized in plasma membranes where the degree of purification is highest, and there is 15–20% contamination independent of the number of gradients.

TABLE V

CONTAMINATION OF PLASMA MEMBRANES BY MEMBRANES FROM THE ENDOPLASMIC RETICULUM AND FROM LYSOSOMES

The figures shown represent the percentage of activity found in plasma membranes calculated from the activity of the original lysate.

Enzyme marker	*Cell lines*	
	MF2 S	*ME2*
UDPase	5	–
Glucose-6-phosphatase	–	20
Alkaline phosphatase	2	15
NADH: cytochrome *c* reductase	–	–
Acid phosphatase	10	8

The high level of contamination can be explained either by the existence of different proteins in the plasma membranes which have the same activity as those of the reticulum membranes[10], or by a density of 1.14–1.16 for certain reticular membranes, or by a structural relationship between the plasma membranes and the reticulum. This last hypothesis is apparently confirmed by the fact that the quantity of contaminating membranes remains constant when the density of the plasma membranes is changed by separation on a second gradient[8].

We are therefore led to suppose that in the case of epithelioid cells a fraction of the endoplasmic reticulum is tightly bound to the plasma membrane, whereas in MF2 cells such an association does not exist.

In addition, when a reticular enzyme such as NADH: cytochrome *c* reductase is largely solubilized it is shown that the residual activity remains in the surface membrane fraction and is not separated from it on a further gradient. In the case where there is an association between plasma membranes and endoplasmic reticulum it is possible that the enzyme activities may be protected against solubilization.

Whereas lysosomes may be completely eliminated from the surface membrane fractions of MF2 cells[8] the level of contamination for ME2 and MF2 cells remains 10% or more. This is not considerable in view of the fact that lysosomes have a density of flotation of 1.14–1.16 (ref. 11).

In view of these results, it appears that the factor of contamination is not necessarily unfavorable, but can be used to obtain further information about the system, such as the localization of different enzymes in the cell, the structural relationship between the contaminant and the material contaminated, and the purity of the material isolated according to different markers. If one examines closely the distribution of reticular enzymes as a function of different cell type, certain points arise:

In MF2 cells they are concentrated at the level of the nuclei (40–60% of initial activities). In ME2 cells glucose-6-phosphatase and alkaline phosphatase activities are highest in nuclear fractions (60%, 15%) and in plasma membranes (20%, 15%).

In MF2 S cells UDPase is found in nuclear fractions (60%) but also in all the other fractions (38%), while alkaline phosphatase is principally found in a band of density 1.20 (52%).

In summary, these enzyme activities are associated with the nuclear fraction on MF2 cells, with the dense reticulum in MF2 S, and in both nuclear and plasma membrane fractions in ME2 cells.

One may ask whether a topological study of the activities of the reticulum would not give some information about functional regulation.

Cell confluence, which has been studied for lines MF2 and ME2, produces very different effects in the different cells types. In the case of MF2, at cell concentrations of 50000 or 200000 cells/cm^2 the enzyme activities do not change. If the cell density of the ME2 epithelioid cells varies from 40000–80000 cells/cm^2, the plasma membrane markers lose 90–99% of their initial activity while activities of the reticulum are not much affected. Four hypotheses can be proposed to explain this phenomenon: (1) The plasma membranes of ME2 cells are more fragile at confluence, and during preparation the osmotic shock results in solubilization or inactivation of marker enzymes. (2) The modifications on membrane structure when two cells are in contact result in a masking of the enzyme which no longer binds substrate. (3) The enzyme is inactivated *in situ*. (4) Synthesis of the enzyme is halted.

Since MF2 cells do not exhibit this phenomenon even at very high cell concentrations (limited only by autolysis) they provide a good system for comparison. The question is what functions are different in the two cells lines MF2 and ME2: They may not possess the same surface receptors (qualitatively and quantitatively) as has been postulated by Inbar and Sachs[12] and Burger[13] or, with the same receptors, the signal of inhibition of growth at a certain cell density may necessitate the transmission of this signal at the membrane level. Thus, transformed cells differ from normal cells not in their acquisition of certain functions but in their loss of a regulatory function by which a "stop" signal is transmitted from the plasma membrane to the nucleus.

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