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Levels of membrane marker enzyme activity in normal and RNA and DNA virus-transformed fibroblasts

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SUMMARY

Activities of 5′-nucleotidase, a plasma membrane marker enzyme; esterase, a rough endoplasmic reticulum marker enzyme; and UDPase, a smooth endoplasmic reticulum marker enzyme, were measured in 3T3, RSV-3T3, MSV-3T3 and PY-3T3 cells. 5′-Nucleotidase and esterase activities were significantly elevated in the oncogenic virus-transformed cell lines compared to the 3T3 cells on a per mg protein basis. Activity of UDPase was slightly elevated in the oncogenic virus-transformed cells.

In order for an enzyme to be classified as a membrane marker enzyme it must meet at least three criteria: (1) The enzyme must be rather tightly bound to or associated with the membrane fraction so that solubilization does not occur during fractionation. (2) The enzyme must be present almost exclusively in the membrane fraction that it 'marks'. (3) An assay method must exist which is both simple and specific so that other enzyme activities present in the membranes do not modify the measurement of activity. Three enzymes that more or less fit the above criteria are UDPase (EC 3.6.1.6)[1], described as a smooth endoplasmic reticulum marker enzyme[2,3], esterase (EC 3.1.1)[4], a rough endoplasmic reticulum marker enzyme[3,5], and 5′-nucleotidase (EC 3.1.3.5)[6], a plasma membrane marker enzyme[3,7]. A property these enzymes hold in common in addition to their utility as membrane marker enzymes is that the activity of each is distributed in mitotic cycle synchronized mammalian cells as a peak enzyme in the S period[8].

One focus of oncogenic transformation of mammalian cells is the cell membranes, in particular the plasma membranes. Differences between 'normal' cells and their oncogenically transformed cell counterparts have been noted in such properties as agglutination with various plant lectins, electrophoretic mobility, antigenicity, membrane

structural molecules, and synthesis of membranes[8]. Because of the connection between membranes and oncogenic transformation the present report gives data on membrane marker enzyme activity levels in normal and oncogenic virus-transformed cells.

3T3, a 'normal' established cell line originally derived from mouse embryo fibroblasts; MSV-3T3, an established murine sarcoma virus-transformed cell line; RSV-3T3, an established Rous sarcoma virus-transformed cell line; and PY-3T3, an established polyoma virus-transformed cell line were grown in Dulbecco–Vogt medium *plus* 10% calf serum in monolayer, as previously described[9,10]. Cells were harvested at confluency by scraping, were washed twice with saline, and were immediately homogenized at 4 °C in 20 vol. of 0.1% Triton X-100. This detergent homogenate was used to measure activity of the membrane marker enzymes. Optimal conditions for each assay were determined with the various extracts, and these conditions were used in all experiments. Assays were performed on cell-free extracts rather than on purified enzyme preparations since activity could be selectively lost or enhanced in purification procedures and such assays would not reflect true *in vivo* differences between the various cells.

Protein was determined by the method of Lowry *et al.*[11]. Crystalline bovine serum albumin was used as a standard. Esterase activity in the 0.1% Triton X-100 extracts was determined with *p*-nitrophenyl acetate as substrate by the method of Bier[12,4]. The 5′-nucleotidase activity was assayed with 5′-AMP as substrate by the method of Heppel and Hilmoe[13,6], and the UDPase activity was determined by the method of Plaut[14,1]. Cell numbers were determined by counting in a Coulter Counter Research Model B. Plasma membranes were prepared by the $ZnCl_2$ method of Warren and Glick[15].

The results shown in Table I demonstrate that UDPase, the smooth endoplasmic reticulum marker enzyme, was slightly elevated in the virus-transformed cells compared to the 3T3 cells. Highest elevation (0.4 unit) occurred in the MSV-3T3 and PY-3T3 cells; levels of UDPase in the RSV-3T3 cells were essentially equivalent to the levels in the 3T3 cells, on a mg protein basis. On a per cell basis UDPase activity was elevated in each of the virally transformed cell lines.

Esterase, the rough endoplasmic reticulum marker enzyme, was significantly elevated in the oncogenic virus-transformed cells compared to the 3T3 cells. In the RSV-3T3 and PY-3T3 cells levels of esterase were essentially double those found in the 3T3 cells (Table I) while in the MSV-3T3 cell lines esterase activity was three times that of the 3T3 cells, on a mg protein basis. The elevation was even greater when the results were expressed per cell.

5′-Nucleotidase, the plasma membrane marker enzyme, also had significantly elevated activity in the virus-transformed cell lines. As noted for the other two enzyme activities, the highest elevation was found for 5′-nucleotidase in the MSV-3T3 cells; both the RSV-3T3 and PY-3T3 cell extracts had more than twice the 5-nucleotidase activity on a per mg protein basis than the 3T3 cells; on a cell basis the elevated levels in the virally transformed cells was slightly more pronounced.

The data given in Fig. 1 indicate that the three enzyme activities were essentially linear with added cell detergent homogenate up to 200 μg of homogenate protein for the

TABLE I

ACTIVITY LEVELS OF THREE MEMBRANE MARKER ENZYMES IN NORMAL AND ONCOGENIC VIRUS-TRANSFORMED CELLS

Values are means from 11 to 21 independent observations ± 1 S.D. Experiments were performed as described in the text and approximately 0.1 mg of cell homogenate protein was utilized per assay.

Cell line	*Activity (μmoles product formed/h per mg cell homogenate protein)*			*Activity (μmoles product formed/h per 10^6 cells)*		
	UDPase	*Esterase*	*5'-Nucleotidase*	*UDPase*	*Esterase*	*5'-Nucleotidase*
3T3	4.3 ± 0.07	4.6 ± 0.41	1.21 ± 0.10	5.1 ± 0.05	5.4 ± 0.35	1.59 ± 0.14
RSV-3T3	4.4 ± 0.09	9.8 ± 0.43	3.09 ± 0.09	5.9 ± 0.67	13.1 ± 2.01	4.11 ± 0.38
MSV-3T3	4.7 ± 0.18	14.2 ± 0.60	3.32 ± 0.14	6.2 ± 0.67	19.0 ± 3.39	4.54 ± 0.67
PY-3T3	4.7 ± 0.09	9.7 ± 0.22	2.78 ± 0.10	6.1 ± 0.39	14.6 ± 1.34	4.23 ± 0.56

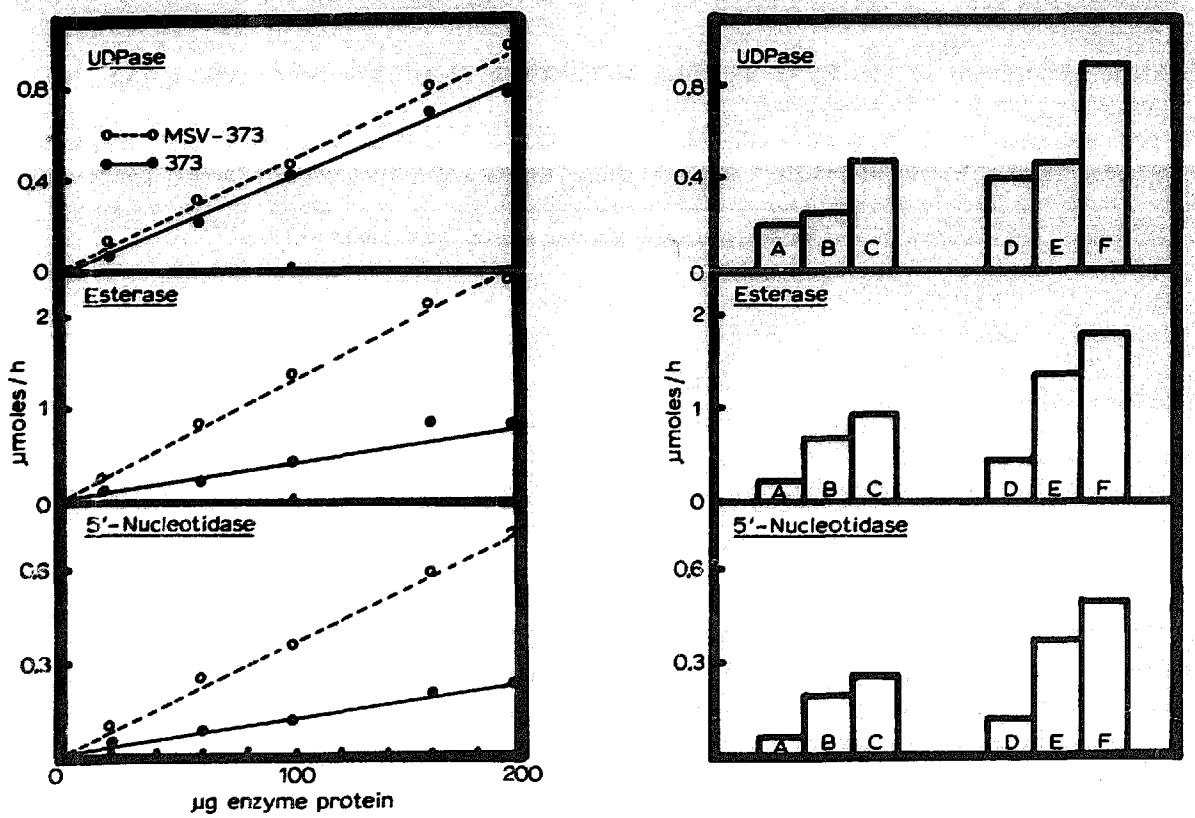

Fig. 1. Activity of three membrane marker enzymes in normal and MSV-transformed cells as a function of amount of cell extract. Experiments were performed as given in text.

Fig. 2. Activity of three membrane marker enzymes in normal and MSV-transformed cells in mixed assays. Experiments were performed as given in the text. A = 50 µg 3T3 extract, B = 50 µg MSV-3T3 extract, C = 50 µg 3T3 extract + 50 µg MSV-3T3 extract, D = 100 µg 3T3 extract, E = 100 µg MSV-3T3 extract, and F = 100 µg 3T3 extract + 100 µg MSV-3T3 extract as enzyme source.

3T3 and MSV-3T3 cell lines; similar results were obtained for the RSV-3T3 and PY-3T3 cell lines. The data in Fig. 2 represent data from mixed enzyme assays performed to determine if factors such as inhibitors or activators were responsible for the differences in activities between the normal and oncogenic virus cell homogenates. The data show that for the MSV-3T3 and 3T3 cell homogenates activity was essentially additive, ruling out possibilities of factors other than activity being responsible for the differences reported in Table I. Similar results were obtained when either RSV-3T3 cell or PY-3T3 cell homogenates were mixed with 3T3 cell homogenates.

The data presented in Table II show that even in plasma membranes prepared with high concentrations of Zn^{2+}, high specific activities of 5′-nucleotidase were found in all of the cell lines studied; this points to the utility of the enzyme as a marker and to the fact that it is present in the same fraction (surface membrane) after transformation. The results in the table also clearly demonstrate that the activity in the plasma membranes of the virally transformed cells is greatly elevated over that of the plasma membranes of the non-transformed control cells. In this regard it is of extreme interest that it has recently[16] been demonstrated that 5′-nucleotidase activity is much greater in MF_2 cells (line of plasmo-

TABLE II

5'-NUCLEOTIDASE ACTIVITY OF PLASMA MEMBRANE FRACTION OF NORMAL AND ONCOGENIC VIRUS-TRANSFORMED CELLS

Values are means ± S.D. from eight independent observations. The plasma membrane fraction prepared by the method of Warren and Glick[15] was substituted for the cell homogenate in these experiments.

Cell line	*5'-Nucleotidase activity (μmoles/h per mg plasma membrane protein)*
3T3	52.9 ± 8
RSV-3T3	165.8 ± 30
MSV-3T3	189.9 ± 11
PY-3T3	120.5 ± 5

cytoma MOPC 173), which are not contact inhibited, than in ME_2 cells (also derived from MOPC 173), which are contact inhibited. Also when these cells come into contact levels of 5'-nucleotidase sharply decrease[16]. Thus the increased levels of activity of 5'-nucleotidase reported herein for both total cell homogenates and plasma membranes of virally transformed cells may be the result of major changes in the plasma membrane of the transformed cells which are associated with loss of contact inhibition and release of the cells from growth restraints.

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