Protein kinase C translocation in relation to proliferative state of C3H 10T1/2 cells

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Protein kinase C (PKC) activity of cytosol and membrane fractions of 10T1/2 cells was studied. In cytosol of fast growing cells PKC activity was found in material eluted with 0.150 M NaCl whereas in membrane fractions activity was eluted with 0.065 and 0.150 M NaCl. In the membrane fraction of confluent cells, in contrast to cytosol, very low PKC activity was observed. The translocation pattern of PKC activity eluted with 0.065 M NaCl may be associated with proliferation.

Protein kinase C *Proliferation* *Contact inhibition*

1. INTRODUCTION

Nishizuka [1] demonstrated that protein kinase C (PKC) is essential in transduction of extracellular signals for cell proliferation and function. Cell receptor stimulation by some exogenous factors such as PDGF and fetal calf serum (FCS) leads to fast phosphoinositide turnover, with subsequent hydrolysis of phosphatidylinositol 4,5-bisphosphate to diacylglycerol and inositol 1,4,5-trisphosphate for PKC activation and Ca^{2+} mobilization [1–3].

Here, we investigated PKC activity in C3H (10T1/2) cells during their fast phase of growth and when the cells had become contact-inhibited, assuming that cessation of the growth may be associated with a variation of the activity of the studied enzyme.

In the system we used, logarithmic and stationary 10T1/2 cells were stimulated by the addition of 10% FCS following 12 h cultivation in serum-free medium.

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2. MATERIALS AND METHODS

2.1. *Cell culture*

C3H 10T1/2 fetal mouse fibroblasts from C. Heidelberger's laboratory were grown in MEM supplemented with 10% FCS. For experiments 1000 cells were seeded into each petri dish. Cells were allowed to grow for 3 days to attain the logarithmic phase and 9–10 days for the stationary phase. After 12 h cultivation in serum-free medium, cultures were challenged with 10% FCS supplemented medium.

2.2. *Preparation of cytosol and membrane fractions*

10 min after the medium was changed to one supplemented with 10% FCS. Cells were scraped off, rinsed with PBS and then centrifuged for 10 min at 100 × *g*. The cell pellet was resuspended and homogenized in buffer A (20 mM Tris-HCl, pH 7.5; 30 mM 2-mercaptoethanol, 2 mM EDTA, 0.5 mM EGTA, 2 mM PMSF) and the homogenate was centrifuged at 100000 × *g* for 1 h at 4°C. The supernatant was used as the cytosol fraction and the pellet was rehomogenized in 0.1%

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Triton X-100 containing buffer A, and centrifuged at 100000 × *g* for 1 h. The supernatant was used as the particulate or membrane fraction. Protein determinations were performed by the microassay of Bradford [4].

2.3. *DEAE-cellulose chromatography*

Each supernatant was applied to a DE 52 cellulose (0.9 × 2.0 cm) column equilibrated with buffer A. Elution was performed with a linear 0.01–0.30 M NaCl gradient in buffer A.

2.4. *PKC assay*

PKC was assayed by measuring the incorporation of ^{32}P into histone from [^{32}P]ATP. The reaction mixture contained 20 mM Tris-HCl (pH 7.5),

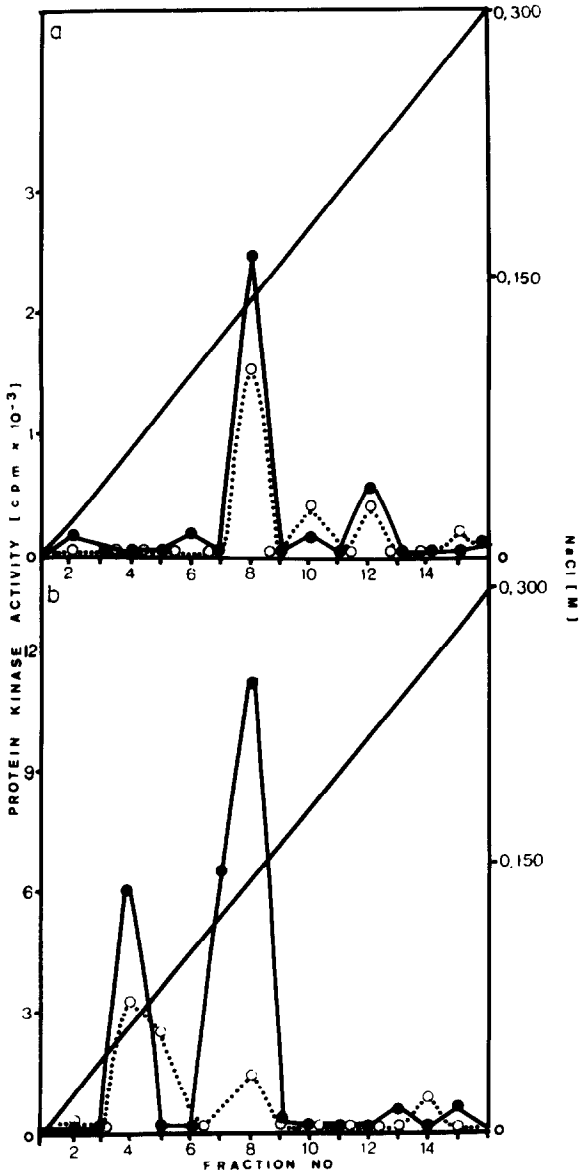

Fig.1. Protein kinase C activity in fast growing 10T1/2 cells in the presence (●——●) or absence (○---○) of phosphatidylserine; (a) cytosol, (b) membrane.

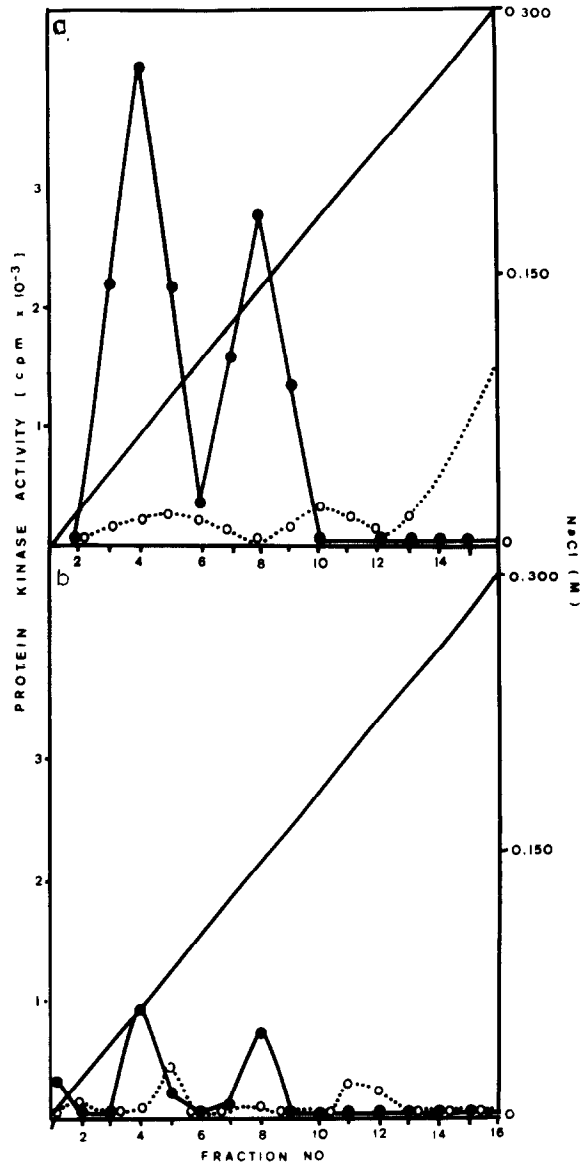

Fig.2. Protein kinase C activity in confluent 10T1/2 cells in the presence (●——●) or absence (○---○) of phosphatidylserine; (a) cytosol, (b) membrane.

4 mM Mg acetate, 15 μg H1 histone. Phosphatidylserine (10 μg), TPA (50 μg) and Ca^{2+} (0.07 mM) were also added, depending on the experimental conditions. After incubation for 3 min at 30°C the reaction was stopped by the addition of 25% trichloroacetic acid and the precipitates collected and washed on GF/C cellulose filters.

3. RESULTS

3.1. *PKC activity patterns*

In 10T1/2 cells PKC activity was found in both cytosol and cell membrane fractions. The enzyme distribution, however, showed variability depending on the tissue culture conditions. The cytosol fraction of fast growing cells displayed one peak of PKC activity material eluted with 0.150 M NaCl, whereas in the membrane fraction two peaks of activity were observed which were eluted with 0.065 and 0.150 M NaCl. In confluent 10T1/2 cells the PKC activity of cytosol was found in two peaks eluted with the same gradient density (0.065 and 0.150 M NaCl). The membrane fraction of confluent cells also showed two peaks of PKC activity, but at a very low level. These findings are displayed in figs 1 and 2.

3.2. *PKC activity*

To compare the results obtained we calculated the activity of PKC (pmol ^{32}P/min per mg protein) in membrane and cytosol fractions of growing and confluent 10T1/2 cells. In the membrane fraction of confluent cells PKC activity in both peaks was 10–25-times lower than in the membrane of fast growing cells. The cytosol fraction of fast growing cells displayed activity only in material eluted with 0.150 M NaCl. The results are presented in table 1.

Table 1

Protein kinase C activity (pmol ^{32}P/min per mg protein) in cytosol and membrane fractions of fast growing and confluent C3H 10T1/2 cells

	Cytosol		Membrane	
	0.065 M NaCl eluted	0.150 M NaCl eluted	0.065 M NaCl eluted	0.150 M NaCl eluted
Fast growing	–	469	583	1989
Confluent	300	206	15	28

Cytosol and membrane fractions were obtained as described in section 2. PKC activity was measured in materials eluted with 0.065 and 0.150 M NaCl from DE 52 cellulose

4. DISCUSSION

Extensive proliferation of 10T1/2 cells took place during the early period after initiation of cultures, cells later became confluent and cessation of multiplication was observed [5,6]. This growth pattern would suggest that the signalling device for proliferation in cell membranes is blocked or that signal transduction is inefficient.

To prove this hypothesis we studied PKC, the enzyme activated by diacylglycerol, which is produced in membranes following external signal inducing phosphoinositide turnover [1].

In confluent cells the majority of the activity was found in the cytoplasm whereas only 5–10% was in the membrane. Both fractions possessed activity eluted with 0.065 and 0.150 M NaCl. In fast growing cells in turn only one peak of activity was present in the cytoplasm and two in the membrane fraction. The results described suggest that the pattern of PKC translocation from the cytoplasm to the membrane can be associated with the proliferative ability of cultured 10T1/2 cells. PKC translocation in a variety of cells has been reported [7,8].

Also the lack of a detectable amount of PKC activity in membranes with a subsequent occurrence after phorbol stimulation of confluent 10T1/2 cells has been described [9].

In the present experiments the most important finding is that PKC eluted with 0.065 M NaCl was completely translocated to membranes of fast growing cells. Considering the biological role of 0.065 M NaCl eluted PKC it appears that it may be responsible for proliferative signal transduction. Evidence from the work of Chida and co-workers [9] showing the reoccurrence of PKC activity in membranes of confluent 10T1/2 cells after TPA stimulation and the lack of PKC translocation found here strongly suggest that the production of diacylglycerol is reduced, perhaps resulting from blocked cell surface receptors by neighboring counterparts. The possibility that during confluency the cells may develop or enhance a mechanism

protecting phosphoinositide turnover cannot be excluded. Without considering the fine mechanism we can postulate that one of the important factors associated with contact inhibition is that which leads to inhibition of PKC translocation to membranes.

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