CHANGES IN PHOSPHOLIPID POLAR HEAD GROUP TURNOVER IN SV40-TRANSFORMED HAMSTER FIBROBLASTS

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1. Introduction

Cell transformation by oncogenic viruses is accompanied by modifications of membrane structure and functions [1,2]. As major constituents of the cell membrane, phospholipids might play a role in these processes.

Studies on phospholipid composition showed that the proportions of the major phospholipids PC, PE and PI were not modified by viral transformation [3,4]. Only a decrease in sphingomyelin, a typical membrane phospholipid has been described [5,6].

Incorporation of [^{32}P]phosphate pointed out some modifications of phospholipid metabolism at the onset of contact inhibition, especially concerning the turnover rates of PC and PE [3,7]. An increase in PI polar head group turnover in SV40-transformed mouse fibroblasts has been described [8]. We have demonstrated enhanced phospholipid methylation by viral transformation in hamster cells [9]. Thus, phospholipid metabolism appeared to be related to cell proliferation and contact inhibition.

This work compares the turnover of phospholipid polar head groups in established and SV40-transformed hamster fibroblasts, by incorporation of specific precursors such as [^{14}C]inositol, [^{14}C]choline and [^{14}C]-ethanolamine. It was demonstrated that incorporation of inositol into PI and of ethanolamine into PE were enhanced in transformed cells. In contrast, incorporation of choline into PC and SM was decreased in these cells.

Abbreviations: SM, sphingomyelin; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol

2. Materials and methods

2.1. *Cell culture*

The two cell lines studied were EHB cells, established hamster fibroblasts, and EHSVi cells, SV40-transformed hamster fibroblasts [10]. EHB cells displayed several characteristics of normal cells such as morphology, contact inhibition, growth rate and adherence to support [5].

Cells were seeded at $3 \times 10^4/cm^2$ in 25 cm^2 Corning flasks, with 5 ml Eagle's Minimum Essential Medium supplemented with 10% foetal calf serum (Gibco). All experiments were performed in exponential growth phase, 2 days after seeding.

2.2. *Incorporation of labelled precursors*

Phospholipids were labelled with 0.1 μCi/ml [U-^{14}C]inositol 278 mCi/mmol, 0.5 μCi/ml [*methyl*-^{14}C]choline 52 mCi/mmol, 0.5 μCi or 5 μCi/ml [2-^{14}C]-ethanolamine 44 mCi/mmol from Amersham. After incubation at 37°C from 1–6 h, cells were washed 3 times with NaCl (9 g/l) harvested with a rubber policeman, centrifuged and resuspended in NaCl (9 g/l).

Chromatography of phospholipids was performed after direct application of cell suspensions on silica gel plates Schleicher and Schüll F 1500, as in [11]. The solvent system was chloroform/methanol/H_2O (65:25:4, by vol.). We have checked that phospholipid extraction was total in these conditions. Phospholipids were identified with known standards, visualized by autoradiography, cut out and counted by liquid scintillation. Results are expressed in mol precursor incorp./mol phospholipid.

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3. Results

Fig.1 shows that incorporation of inositol into PI was ~2-times more rapid in transformed cells. Labelled PI appeared inside the cell as soon as 1 h after introduction of the precursor.

Introduction of [^{14}C]choline in the medium was followed by appearance of labelled PC and SM. In fig.2a, it can be seen that choline incorporation into PC was markedly lowered (X2) by viral transformation. The same conclusion can be drawn for SM synthesis from [^{14}C]choline (fig.2b). However, a difference in the kinetics of appearance of labelled PC and SM must be noted: SM showed a marked delay in incorporation of [^{14}C]choline. This low turnover rate of SM polar head group has been described in cultured fibroblasts by [^{32}P]phosphate incorporation [5,12]. The decreased incorporation of choline into SM observed in transformed cells might be a consequence of the decreased incorporation of the precursor into PC, since it was demonstrated that SM was synthesized by transfer of phosphorylcholine from PC to ceramide [13,14].

Results obtained with [^{14}C]ethanolamine are given in fig.3. The presence of labelled PE was observed 1 h after labelling. A marked increase of [^{14}C]ethanolamine incorporation into PE can be noted in transformed cells (X2).

Enhanced PE methylation has been described in EHSVi transformed cells, using [*methyl*-^{14}C]methionine as precursor of PC [9]. Thus, the question rises as to whether the increased incorporation of ethanolamine into PE observed in these cells is related to the enhanced PE methylation. Using a greater concentration of [^{14}C]ethanolamine, we were able to detect incorporation of this precursor into PC, most probably by methylation of PE. Incorporation of ethanolamine into PC was ~2-fold increased in transformed cells (table 1).

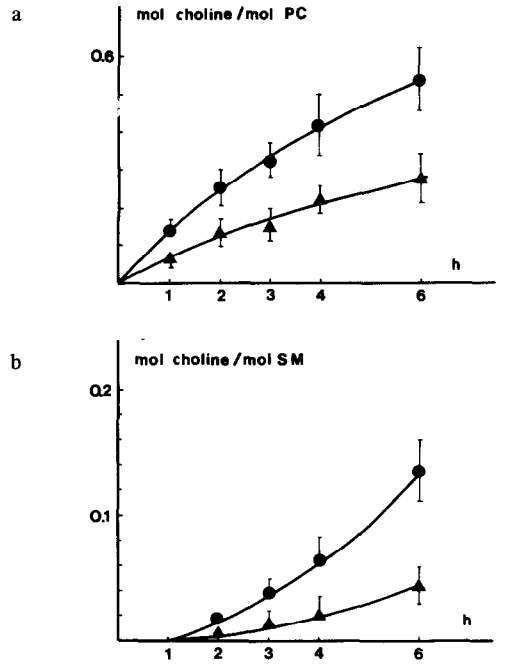

Fig.2. Kinetic of [*methyl*-^{14}C]choline incorporation into PC (a) and SM (b) by established (•) and SV40-transformed cells (▲). Cells were in exponential growth phase and labelling was performed with 0.5 μCi/ml of choline 52 mCi/mmol; mean of 3 expt ± SD.

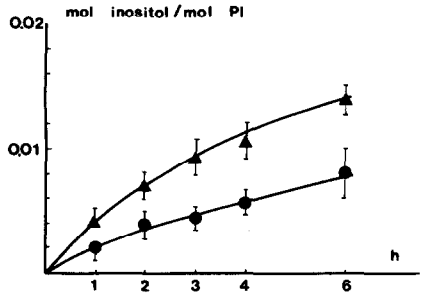

Fig.1. Kinetic of [U-^{14}C]inositol incorporation into PI by established cells EHB (•) and SV40-transformed cells EHSVi (▲). Cells were in exponential growth phase and labelling was performed with 0.1 μCi/ml inositol 278 mCi/mmol; mean of 3 expt ± SD.

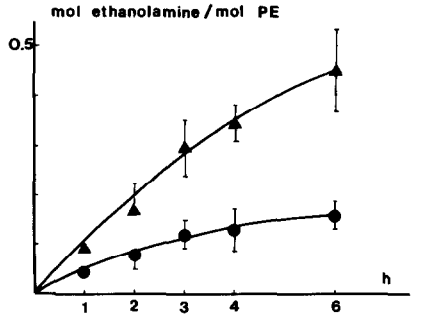

Fig.3. Kinetic of [2-^{14}C]ethanolamine incorporation into PE by established (•) and SV40-transformed cells (▲). Cells were in exponential growth phase and labelling was performed with 0.5 μCi/ml of ethanolamine at 44 mCi/mmol; mean of 3 expt ± SD.

Table 1
Incorporation of ethanolamine into PC in established (EHB) and SV40-transformed hamster fibroblasts (EHSVi)

Incorp. (h)	Ethanolamine incorporated into PC (mol . 10^{-3}/mol PC)	
	EHB	EHSVi
2	1.9 (1.5–2.3)	4.1 (3.7– 4.5)
4	3.3 (3.0–3.6)	6.1 (5.5– 6.7)
6	4.8 (4.3–5.3)	13.7 (12.5–14.9)

Cells were labelled with 5 μCi/ml [2-^{14}C]ethanolamine; mean of 2 expt (range)

4. Discusssion

Incorporation of [^{14}C]inositol pointed out a more rapid turnover of PI polar head group in transformed cells. This result is consistent with the data in [5] or [8] from [^{32}P]phosphate. The metabolism of PI seems to be related to cellular activation by several mechanisms [15]. Turnover rate of PI increases in lymphocytes stimulated by lectins [16] or fibroblasts stimulated by serum addition [17]. Enhanced PI turnover was observed when Ca^{2+} flux [18] or microtubules movement [19] were triggered. But the significance of increased PI turnover remains unclear.

In exponential growth phase, transformed cells incorporated more rapidly ethanolamine into PE and more slowly choline into PC and SM. Changes in turnover rates of PC and PE polar head groups, studied with [^{32}P]phosphate, have been described at the onset of contact inhibition in 3T3 cells [3] and in mouse embryo cells [7]: synthesis of PC was enhanced while that of PE was decreased. But differences in turnover rates of PC and PE in normal and transformed cells on exponential growth phase have not been reported, except in the 2 studied cell lines [5]. However, careful examination of the results in [3] shows that the transformed Py 3T3 cells incorporated much less [^{32}P]phosphate into PC than their normal counterparts.

Very few works have been concerned with regulation of phospholipid synthesis. The importance of CDP–choline pool size in the synthesis of PC was described in [20,21]. Evaluation of intracellular pools of each metabolic precursor, such as choline, choline–phosphate, CDP–choline, in the case of choline incorporation, will be useful to elucidate the phenomenon.

The rapid incorporation of ethanolamine into PE observed in transformed cells is accompanied by an increase of PE methylation, demonstrated by incorporation of either [*methyl*-^{14}C]methionine [9] or [^{14}C]ethanolamine (table 1) as PC precursor. The transmethylation pathway of PC synthesis is only a minor pathway compared with the CDP–choline pathway [22,23]. We observed in table 1 that the radioactivity recovered in PC with [^{14}C]ethanolamine only represents 1% of the radioactivity of PE. Thus, assuming that transformed cells synthesized 2-fold more PE from ethanolamine than normal cells, not all the newly synthesized PE was transmethylated into PC, and enhanced degradation of PE molecules most likely occurred in these cells.

Here, lipid analysis was performed with whole cells. Cell fractionation into cellular membranes, microsomes, lysosomes prior to lipid analysis would be interesting in order to bring further light on phospholipid metabolism. The observed differences in PC and PE metabolism lead to the idea of local differences in PC/PE ratio in the membranes of normal and transformed cells. These local differences have already been shown in SV40-transformed murine cells [24]. Since the ratio PC/PE is determinant for cell-substrate adherence [25,26], the observed modifications in phospholipid metabolism might be in relation with alterations of cellular adhesive properties induced by viral transformation.

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References

[1] Foster, D. O. and Pardee, A. B. (1969) J. Biol. Chem. 244, 2675–2681.
[2] Hatanaka, M. and Hanafusa, H. (1970) Virology 41, 647–652.
[3] Cunningham, D. D. (1972) J. Biol. Chem. 247, 2464–2470.
[4] Howard, B. V. and Kritchevsky, D. (1969) Int. J. Cancer, 4, 393–402.
[5] Mazière, C. and Rampini, C. (1975) Biochimie 57, 1189–1196.
[6] Perkins, R. G. and Scott, R. E. (1978) Lipids 13, 653–657.

[7] Diringer, H. and Koch, M. A. (1973) Biochem. Biophys. Res. Commun. 51, 967–971.
[8] Koch, M. A. and Diringer, H. (1973) Biochem. Biophys. Res. Commun. 55, 305–311.
[9] Mazière, C., Mazière, J. C., Mora, L. and Polonovski, J. (1981) FEBS Lett. 129, 67–69.
[10] Tournier, P., Cassingena, R., Wicker, R., Coppey, J. and Suarez, H. (1967) Int. J. Cancer 2, 117–132.
[11] Dosado, E. A., Wittsie, A. and Snyder, F. (1976) J. Lipid Res. 17, 285–288.
[12] Kulas, H. P., Marggraf, W. D., Koch, M. A. and Diringer, H. (1972) Z. Physiol. Chem. 353, 1755–1760.
[13] Diringer, H. and Koch, M. A. (1973) Z. Physiol. Chem. 354, 1661–1665.
[14] Ullman, M. D. and Radin, N. S. (1974) J. Biol. Chem. 249, 1506–1512.
[15] Michell, R. H. (1975) Biochim. Biophys. Acta 415, 81–147.
[16] Fischer, D. B. and Mueller, G. C. (1971) Biochim. Biophys. Acta 248, 434–448.
[17] Pasternak, C. A. (1972) J. Cell. Biol. 53, 231–234.
[18] Fain, J. and Berridge, M. J. (1979) Biochem. J. 178, 45–48.
[19] Kirazov, E. P. and Lagnado, J. R. (1977) FEBS Lett. 81, 173–178.
[20] Choy, P. C., Paddon, H. B. and Vance, D. E. (1980) J. Biol. Chem. 255, 1070–1073.
[21] Vance, D. E., Trip, E. M. and Paddon, H. B. (1980) J. Biol. Chem. 255, 1064–1069.
[22] Ketyal, S. L. and Lombardi, B. (1976) Lipids 11, 513–516.
[23] Trewhella, M. A. and Collins, F. D. (1973) Biochim. Biophys. Acta 296, 51–61.
[24] Cathcart, M. A. and Culp, L. A. (1979) Biochemistry 18, 1167–1176.
[25] Hale, A. H., Pessin, J. E., Palmer, F., Weber, M. J. and Glaser, M. (1977) J. Biol. Chem. 252, 6190–6200.
[26] Portoukalian, J., Bugand, M., Zwingelstein, G. and Precausta, P. (1977) Biochim. Biophys. Acta 489, 106–118.