Volume 247, number 2, 353-357 FEB 07060 April 1989

Expression of protein kinase C subspecies in human leukemia-lymphoma cell lines

Satoru Sawamura, Katsuhiko Ase, Nicola Berry, Ushio Kikkawa, Patricia G. McCaffrey*, Jun Minowada* and Yasutomi Nishizuka

Department of Biochemistry, Kobe University School of Medicine, Kobe 650 and **Fujisaki Cell Center, Hayashibara Biochemical Laboratories, Inc., Okayama 702, Japan*

Received 3 March 1989

Expression of protein kinase C (PKC) subspecies was studied in various human leukemia-lymphoma cell lines. The PKC in most cell lines examined was resolved into two major fractions corresponding to type II (β-sequence) and type III (α-sequence) PKC of the rat brain. The amounts of these two subspecies greatly varied among the cell lines. Type I PKC (γ-sequence) was expressed in none of the cell lines tested, but PKCs with undefined structures were frequently detected. The differential co-expression of several PKC subspecies is presumably related to the state of cell differentiation.

Protein kinase C; Differentiation; (Human leukemia-lymphoma cell line)

1. INTRODUCTION

Protein kinase C is generally accepted to play a pivotal role in physiological cellular responses to external signals such as those related to growth promotion and cell differentiation (reviews [1,2]). Molecular cloning and biochemical analysis of this enzyme reveals PKC as a family of multiple subspecies that have similar but distinct structures [2]. Initially, four cDNA clones, α, βI, βII and γ, were isolated, which appear to be conserved among various mammalian species [2]. PKC from the brain tissues is normally resolved into three fractions, type I, II and III, upon hydroxyapatite column chromatography [3-5], which correspond to the enzyme encoded by γ-, β (βI plus βII)- and α-cDNA clone, respectively [4-6]. Some of these PKC subspecies show tissue-specific expression [6-9] and different ontogenic profiles [9,10]. A number of different human leukemia-lymphoma cell lines have been established in suspension culture [11-14]. The application of multiple marker analysis indicates that each cell line consists of a homogeneous and monoclonal population, and that the cells are arrested at a certain stage of differentiation representing distinct differentiation-associated phenotypes, whose characteristics are consistently stable during long-term maintenance [14]. The expression of PKC subspecies in some of the human leukemia-lymphoma cell lines will be described herein.

The data are taken in part from the dissertation that will be submitted by S. Sawamura to Kobe University School of Medicine in partial fulfillment of the requirement for the degree of Doctor of Medical Science

Correspondence address: S. Sawamura, Department of Biochemistry, Kobe University School of Medicine, Kobe 650, Japan

Abbreviation: PKC, protein kinase C

2. MATERIALS AND METHODS

2.1. *Cell lines*

The leukemia-lymphoma cell lines used are assigned to one of the following groups: T-cell leukemia-lymphoma, B-cell leukemia-lymphoma, non-T/non-B cell leukemia, myelomonocytic leukemia and erythroleukemia cell lines [14]. The differentiation stages of T- and B-cell leukemia-lymphoma cell lines are subcategorized as described in [11]. All cell lines were grown as

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

suspension culture in RPMI 1640 medium supplemented with 5% heat-inactivated fetal calf serum at 37°C in a humidified 5% CO_2 atmosphere, and cells in their exponential growth phase were used. The cell lines studied are listed in table 1.

2.2. *Purification and assay of PKC*

PKC in each cell line was partially purified under the conditions specified earlier [15,16]. Briefly, the supernatant of the cells (approx. 5×10^8 cells) was applied to a DE-52 column (1×2.5 cm, Whatman) equilibrated with 20 mM Tris-HCl at pH 7.5, containing 0.5 mM EGTA, 0.5 mM EDTA and 10 mM 2-mercaptoethanol (buffer A). PKC was eluted from the column by 6 ml of buffer A containing 200 mM NaCl, and was applied to a packed hydroxyapatite column (0.78×10 cm, Koken Co., Ltd., Tokyo, Type S), which was connected to a Pharmacia FPLC system and equilibrated with 20 mM potassium phosphate buffer at pH 7.5, containing 0.5 mM EGTA, 0.5 mM EDTA, 10% glycerol and 10 mM 2-mercaptoethanol (buffer B). PKC was eluted by application of an 84-ml linear concentration gradient of potassium phosphate (20–215 mM) prepared in buffer B. Fractions of 1 ml each were collected. Rat brain PKC, a mixture of type I, II and III, was purified as described in [4]. The enzyme was assayed with calf thymus H1 histone as substrate as specified earlier [4].

2.3. *Immunoblot analysis*

Immunoblot analysis was made as described previously [8]. The antibodies employed for the present studies were raised against synthetic oligopeptides which are parts of the deduced amino acid sequences of the PKC subspecies [7,8]. The antibody CKpClβ-a reacts equally with α-, βI-, βII- and γ-subspecies, and the antibodies CKpVlα-a, CKpV5βI-a and CKpV5βII-a react specifically with α-, βI- and βII-subspecies, respectively [7,8].

2.4. *Immunocytochemical analysis*

Immunocytochemical staining of the leukemia-lymphoma cells was carried out as described in [15]. Cells were fixed with 1% paraformaldehyde, cytocentrifuged onto glass slides and permeabilized with 0.3% Triton X-100. After incubation with 1% H_2O_2 in methanol to block endogenous peroxidase, the cells were incubated with antibodies specific to the different PKC subspecies. Immunoreaction was visualized using the biotin-conjugated anti-rabbit antibody and streptavidin-conjugated peroxidase (Biogenex, CA) employing diaminobenzidine as substrate and observed under the light microscope.

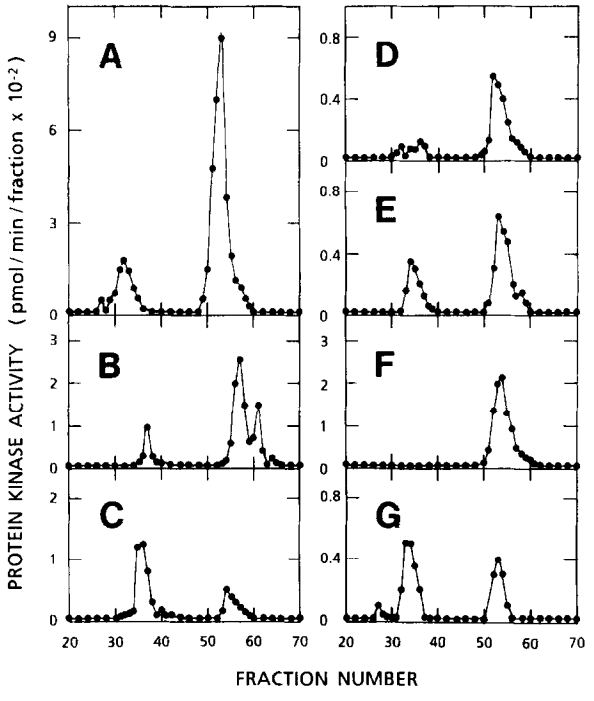

Fig.1. Hydroxyapatite column chromatography of PKC from T-cell, B-cell and non-T/non-B cell leukemia-lymphoma cell lines. The PKC activity of these cell lines was separated by hydroxyapatite column chromatography and enzymatic activity was assayed as described in section 2. (A–C) PKC from T-cell leukemia-lymphoma cell lines, HPB-ALL, MOLT-3 and MOLT-15, respectively; (D–F) PKC from B-cell leukemia-lymphoma cell lines, NALM-6, U-698-M and RAMOS, respectively; (G) PKC from non-T/non-B cell leukemia-lymphoma cell line, KM-3; (●——●) PKC activity.

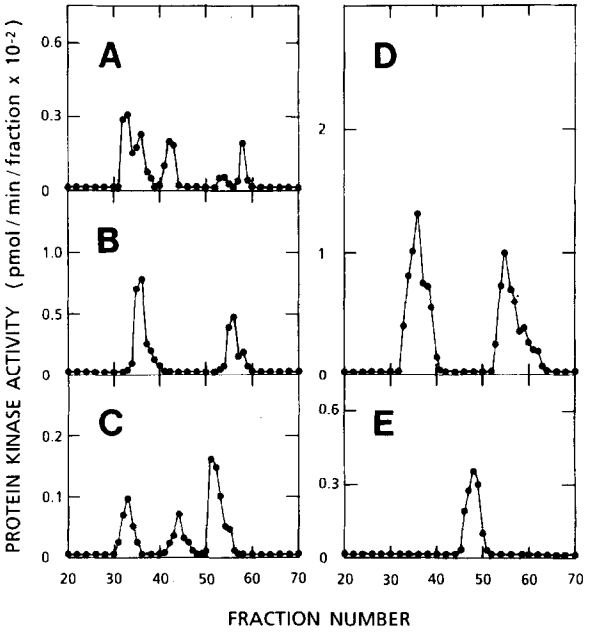

Fig.2. Hydroxyapatite column chromatography of PKC from myelomonocytic leukemia and erythroleukemia cell lines. The PKC activity of these cell lines were separated by hydroxyapatite column chromatography and enzymatic activity was assayed as described in section 2. (A–C) PKC from myelomonocytic leukemia cell lines, KG-1, ML-2 and HL-60, respectively; (D,E) PKC from erythroleukemia cell lines, SPI-802 and HEL, respectively; (●——●) PKC activity.

3. RESULTS

3.1. *Resolution of PKC subspecies*

The PKC of T-cell, B-cell and non-T/non-B cell leukemia-lymphoma cell lines was normally resolved into two major peaks upon hydroxyapatite column chromatography (fig.1). The two enzyme peaks in HPB-ALL, MOLT-3, MOLT-15, NALM-6, U-698-M and KM-3 cells appeared at the concentrations of potassium phosphate that correspond to the elution positions of brain type II (β-sequence) and type III (α-sequence) PKC subspecies [4,5]. In general, T-cell lines contained more PKC than B-cell lines. RAMOS cells have type III but not type II PKC. On the other hand, MOLT-3 cells contain an additional small peak right after the peak of type III, the structure of which remains unknown (fig.1B).

In myelomonocytic leukemia cell lines, the two peaks of PKC were normally detected; KG-1, ML-2 and HL-60 cells contained enzymes corresponding to type II and type III (fig.2). In KG-1 and HL-60 cells additional peaks were found, the kinetic properties of which remain to be defined (fig.2A and C).

In erythroleukemia cell lines, two enzyme peaks corresponding to type II and type III were also detected in SPI-802 cells (fig.2D). However, a single peak of PKC activity was detected in HEL cells, and this enzyme is apparently distinct from type II and type III, and appeared in the fractions 46 to 50 (fig.2E). The PKC activity expressed in these human leukemia-lymphoma cell lines is summarized in table 1. The heterogeneity of PKC described above did not appear simply due to proteolytic artifacts during the purification procedures. All enzyme peaks were dependent on Ca^{2+}, phospholipid and diacylglycerol.

3.2. *Immunochemical analysis of PKC subspecies*

PKC subspecies expressed in T-cell leukemia-lymphoma cell lines were analyzed using antibodies specific to each PKC subspecies, as these cell lines contain large amounts of PKC as described above. Type II and type III PKC fractions of the T-cell leukemia-lymphoma cell lines reacted with the an-

Table 1

Expression of PKC subspecies in human leukemia-lymphoma cell lines

Cell line	Name	Stage	Origin	Specific activity (nmol/min per mg)[e]		
				Type II		Type III
T-cell leukemia-lymphoma cell lines	HPB-ALL	T II	ALL[a]	0.81		3.13
	MOLT-3	T III	ALL	0.19		1.02
	MOLT-15	T IV	AMoL[b]	0.44		0.18
B-cell leukemia-lymphoma cell lines	NALM-6	Pre B	ALL	0.05		0.23
	U-698-M	B I	Lymphoma	0.12		0.25
	RAMOS	B I	Burkitt's			0.93
Non-T/non-B cell leukemia cell line	KM-3		ALL	0.19		0.12
Myelomonocytic leukemia cell lines	KG-1		AML[c]	0.12	0.05[f]	0.03
	ML-2		AML	0.22		0.13
	HL-60		APL[d]	0.03	0.02[f]	0.06
Erythroleukemia cell lines	SPI-802		ALL	0.56		0.47
	HEL		Erythroleukemia		0.12[g]	

[a] ALL, acute lymphoblastic leukemia
[b] AMoL, acute monoblastic leukemia
[c] AML, acute myelomonocytic leukemia
[d] APL, acute promyelomonocytic leukemia
[e] Protein kinase activity/mg protein in crude supernatant
[f] Protein kinase activity appears in fractions 41 – 46 as shown in fig.2A and C
[g] Protein kinase activity appears in fractions 46 – 50 as shown in fig.2E

tibody, CKpClβ-a, and showed an approximate molecular mass of 80 kDa (fig.3). This antibody recognizes a part of the amino acid sequence found almost commonly in rat brain type I (γ-sequence), type II (βI- and βII-sequence) and type III (α-sequence) PKC. Most of the type II and type III PKC fractions of the other cell lines tested also showed similar 80 kDa band. Immunoblot analysis using antibodies CKpV5βI-a and CKpV5βII-a, which can distinguish the βI- and βII-sequence, indicated that the type II PKC fractions of T-cell leukemia-lymphoma cell lines contained predominantly the βII-subspecies, and only a small quantity of the βI-subspecies.

The expression of PKC subspecies in some human leukemia-lymphoma cell lines was investigated further by immunocytochemical staining with polyclonal antibodies specific to the α- and βII-sequence (fig.4). The staining was detected in the cytoplasm, although some membrane localization was noted within the T-cell leukemia-lymphoma cell lines, notably in MOLT-15.

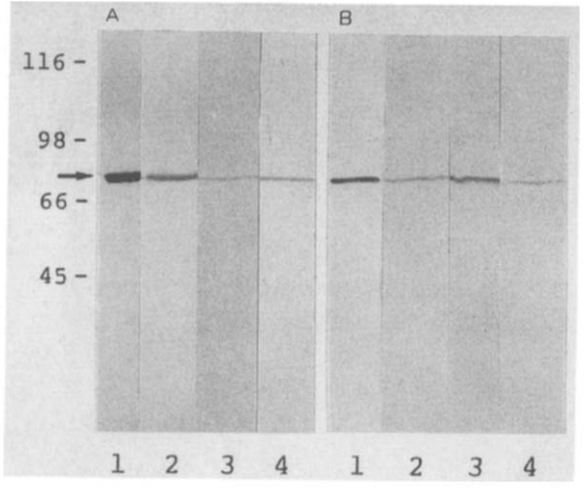

Fig.3. Immunoblot analysis of PKC fractions from T-cell leukemia-lymphoma cell lines. PKC fractions of hydroxyapatite column chromatography were subjected to immunoblot analysis using a polyclonal antibody, CKpClβ-a as described in section 2. (A, B) immunoblot analysis of type II and type III PKC fractions, respectively. Lanes: 1, PKC from rat brain; 2–4, PKC from T-cell leukemia-lymphoma cell lines, HPB-ALL, MOLT-3 and MOLT-15, respectively. Arrow indicates the position of PKC. Molecular mass markers used are shown in kDa: 116, β-galactosidase; 98, phosphorylase *b*; 68, bovine serum albumin; 45, ovalbumin.

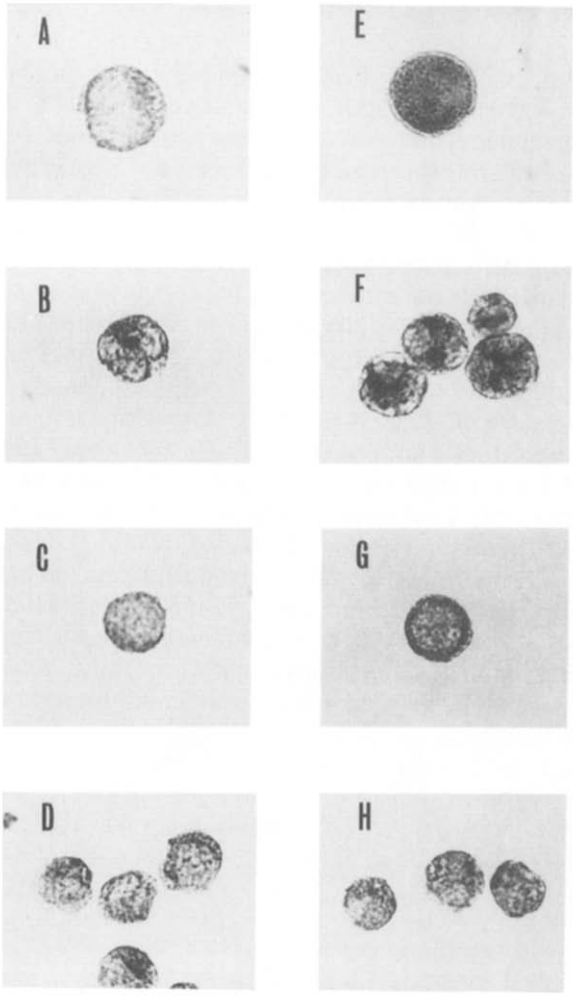

Fig.4. Immunocytochemical staining of T-cell leukemia-lymphoma and non-T/non-B cell leukemia-lymphoma cell lines. T-cell leukemia-lymphoma cell lines, HPB-ALL, MOLT-3 and MOLT-15, and a non-T/non-B cell leukemia-lymphoma cell line, KM-3, were stained with polyclonal antibodies as described in section 2. (A, E) HPB-ALL cells; (B, F) MOLT-3 cells; (C, G) MOLT-15 cells; (D, H) KM-3 cells, respectively; (A–D) stained with the antibody CKpVlα-a; (E–H) stained with the antibody CKpV5βII-a, respectively.

4. DISCUSSION

PKC subspecies show tissue-specific expression [2,6–9]. Type III (α-sequence) is distributed ubiquitously in tissues and cell types so far examined, whereas type I (γ-sequence) is found only in the central nervous tissue [6,8–10]. In the present studies, most of the leukemia-lymphoma cell lines

are shown to co-express both type II and type III, and the ratio of these two subspecies greatly varies with the cell lines tested. The T-cell leukemia-lymphoma cell lines contained a larger amount of PKC activity than the non-T cells. At progressive stages in the preserved maturation of the T-cell leukemia-lymphoma cell lines, the type III enzyme activity was decreased. In contrast, the β-cell leukemia-lymphoma cell lines showed far less activity, and RAMOS cells had no type II enzyme. It has been reported that the RNA transcripts of both βI- and βII-subspecies are detected in rat spleen by in situ hybridization studies [17]. Some of the differentiated B-cells may have both βI- and βII-subspecies. On the other hand, in myelomonocytic leukemia and erythroleukemia cell lines additional minor peaks were found, particularly in KG-1 cells. The PKC in HEL cells, an erythroleukemia cell line, is distinct from both type II and type III PKC. It is possible that these enzymes are encoded by the cDNA clones of PKC, δ, ϵ and ζ, recently identified [18,19], although the precise correlation of these undefined peaks with the cDNA clones of PKC remains unknown. It has been reported that HL-60 cells, a myelomonocytic cell line, express PKC of type I, II and III [20], but the present study indicates that this cell line contains the type II and type III enzymes, and additionally an unidentified subspecies of PKC which is eluted in between. The detailed kinetic properties of this enzyme will be described elsewhere.

Acknowledgements: We thank Sachiko Nishiyama for secretarial assistance. The work in the Department of Biochemistry, Kobe University School of Medicine was supported in part by research grants from the Scientific Research Fund of Ministry of Education, Science and Culture, Japan (1985-1989); Muscular Dystrophy Association U.S. (1986-1989); Yamanouchi Foundation for Research on Metabolic Disorders (1986-1989); Juvenile Diabetes Foundation International, U.S. (1988-1989); Merck, Sharp & Dohme Research Laboratories (1986-1988); Biotechnology Laboratories of Takeda Chemical Industries (1986-1988); Ajinomoto Central Research Laboratories (1986-1988); Meiji Institute of Health Sciences (1986-1988); and New Lead Research Laboratories of Sankyo Company (1988). N.B. is a recipient of grants from the Yamada Science Foundation and the Hayashibara International Cancer Research Fellowship Program.

REFERENCES

[1] Nishizuka, Y. (1986) Science 233, 305-312.
[2] Nishizuka, Y. (1988) Nature 334, 661-665.
[3] Huang, K.-P., Nakabayashi, H. and Huang, F.L. (1986) Proc. Natl. Acad. Sci. USA 83, 8535-8539.
[4] Kikkawa, U., Ono, Y., Ogita, K., Fujii, T., Asaoka, Y., Sekiguchi, K., Kosaka, Y., Igarashi, K. and Nishizuka, Y. (1987) FEBS Lett. 217, 227-231.
[5] Ono, Y., Kikkawa, U., Ogita, K., Fujii, T., Kurokawa, T., Asaoka, Y., Sekiguchi, K., Ase, K., Igarashi, K. and Nishizuka, Y. (1987) Science 236, 1116-1120.
[6] Huang, F.L., Yoshida, Y., Nakabayashi, H., Knopf, J.L., Young, W.S., III and Huang, K.-P. (1987) Biochem. Biophys. Res. Commun. 149, 946-952.
[7] Shearman, M.S., Naor, Z., Kikkawa, U. and Nishizuka, Y. (1987) Biochem. Biophys. Res. Commun. 147, 911-919.
[8] Kosaka, Y., Ogita, K., Ase, K., Nomura, H., Kikkawa, U. and Nishizuka, Y. (1988) Biochem. Biophys. Res. Commun. 151, 973-981.
[9] Yoshida, Y., Huang, F.L., Nakabayashi, H. and Huang, K.-P. (1988) J. Biol. Chem. 263, 9868-9873.
[10] Hashimoto, T., Ase, K., Sawamura, S., Kikkawa, U., Saito, N., Tanaka, C. and Nishizuka, Y. (1988) J. Neurosci. 8, 1678-1683.
[11] Drexler, H.G., Gaedicke, G. and Minowada, J. (1985) Leukemia Res. 9, 209-229.
[12] Drexler, H.G., Gaedicke, G. and Minowada, J. (1985) Leukemia Res. 9, 561-571.
[13] Drexler, H.G., Gaedicke, G., Novotny, J. and Minowada, J. (1986) Cancer 58, 245-251.
[14] Minowada, J. (1983) in: Leukemia (Gunz, F.W. and Henderson, E.S. eds) edn 4, pp. 119-139, Grune & Stratton, New York.
[15] Shearman, M.S., Berry, N., Oda, T., Ase, K., Kikkawa, U. and Nishizuka, Y. (1988) FEBS Lett. 234, 387-391.
[16] Ase, K., Berry, N., Kikkawa, U., Kishimoto, A. and Nishizuka, Y. (1988) FEBS Lett. 236, 396-400.
[17] Brandt, S.J., Niedel, J.E., Bell, R.M. and Young, W.S., III (1987) Cell 49, 57-63.
[18] Ono, Y., Fujii, T., Ogita, K., Kikkawa, U., Igarashi, K. and Nishizuka, Y. (1987) FEBS Lett. 226, 125-128.
[19] Ono, Y., Fujii, T., Ogita, K., Kikkawa, U., Igarashi, K. and Nishizuka, Y. (1988) J. Biol. Chem. 263, 6927-6932.
[20] Makowske, M., Ballester, R., Cayre, Y. and Rosen, O.M. (1988) J. Biol. Chem. 263, 3402-3410.