Altered protein kinase activities of lymphoid cells transformed by Abelson and Moloney leukemia viruses

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Five different types of protein kinase acivities have been evaluated in cell lines from murine lymphomas induced by Abelson leukemia virus (A-MuLV), whose oncogene codes for a tyrosine protein kinase. Such activities were compared with those of normal cells and of cells transformed by Moloney leukamia virus (M-MuLV), lacking oncogene sequences in its genome. While cAMP-dependent protein kinase and cascin kinase-1 do not undergo significant changes, casein kinase-2 rises in both A-MuLV and M-MuLV infected lymphocytes, becoming largely associated with the particulate fraction of transformed cells. Protein kinase-C on the other hand is unchanged in M-MuLV transformed cells but it undergoes a 2–3-fold increment in both soluble and particulate fractions of A-MuLV transformed lymphocytes, which also display high tyrosine protein kinase activity.

Abelson leukemia virus *Moloney leukemia virus* *Protein kinase C* *Casein kinase 2* *Tyrosine protein kinase* *(Thymocyte)*

1. INTRODUCTION

Protein phosphorylation catalyzed by protein kinases and reversed by protein phosphatases has been implicated in the regulation of many cellular processes (reviewed in [1,2]). Expectedly such a network of protein phosphorylations will be affected by that multitude of changes occurring during transformations, so that the identification of protein kinases undergoing marked alterations of activity could either shed some light on the mechanism of oncogenesis or provide helpful diagnostic tools. This would be especially true of cells transformed by viruses coding for tyrosine protein kinases in which an increment of protein phosphorylation at seryl and not only tyrosyl residues has been reported to occur [3,4]. This observation which is suggestive of cascade mechanisms leading to the activation of cellular Ser/Thr specific protein kinases, prompted us to undertake a scrutiny of protein kinase activities in a murine cell line established from thymic lymphomas induced by A-MuLV, whose oncogene expresses a tyrosine protein kinase [5,6]. Such activities were compared with those of normal murine lymphocytes and of a murine T cell lymphoma line induced by M-MuLV, a slow transforming retrovirus from which A-MuLV is derived, lacking however oncogene sequences coding for protein kinases.

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Abbreviations: A-MuLV, Abelson murine leukemia virus; M-MuLV, Moloney murine leukemia virus; cA-PK, cAMP-dependent protein kinase; CK-1 and CK-2, type 1 and 2 casein kinases; LPS, *E. coli* lipopolysaccharide; NP40, nonidet P-40; PK-C, protein kinase-C

2. MATERIALS AND METHODS

The transformed cells used in this study were:

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TA-2, a non-B lymphoma cell line established from thymic lymphomas induced by inoculating intrathymically newborn BALB/c mice with the complex A-MuLV (M-MuLV); ABC-1, a A-MuLV transformed line of pre-B cells, kindly provided by Dr N. Teich [7]; TB-5, a T-cell lymphoma line induced by M-MuLV in BALB/c mice. Controls included normal thymus and spleen cells isolated from BALB/c mice or stimulated in vitro with concanavalin A or LPS, respectively. Cells were cultured in complete medium as described elsewhere [8].

Cells were disrupted and fractionated essentially as in [9] giving rise to a clear cytosol and a particulate fraction. The particulate fraction was partially solubilized at pH 7.5 with non-ionic detergents, essentially as in [10] except for the substitution of Triton X-100 with 1% NP40. Protein kinase assays were carried out on both cytosol and NP40 extract.

Tyrosine protein kinase activity was routinely determined on poly(Glu, Tyr) 4:1 (Sigma) as substrate, as in [10]. Casein kinase activity was assayed on whole casein as in [11], either in the absence or presence of 10 μg/ml heparin, in order to discriminate between type-1 (heparin insensitive) and type-2 (heparin inhibited). Protein kinase-C was evaluated as the Ca^{2+}, phosphatidylserine-dependent phosphorylation of the synthetic peptide Orn_4-Tyr-Gly-Ser-Orn_6-Tyr, at its single seryl residue, under the conditions described in [12]. In some experiments the peptide substrate was replaced by protamine and Ca^{2+} and phospholipid were omitted. cAMP-dependent protein kinase was determined toward the synthetic peptide Arg-Arg-Ala-Ser-Val-Ala, as in [13]. One unit of protein kinase activity was constantly defined as the amount of enzyme transferring 1 pmol P per min to the peptide or protein substrate under standard conditions.

A partial resolution of protein kinase activities was achieved by subjecting the NP40 extracts of particulate fractions to DEAE-Sepharose column chromatography. The columns (1.5 × 4 cm) were equilibrated with 25 mM Hepes buffer, pH 7.0, including also 10% glycerol, 1 mM EDTA, 0.1% NP40, 10 mM mercaptoethanol and 50 μM PMSF. Elution was performed with a 0–0.5 M NaCl linear gradient in the same buffer. 0.9 ml fractions were collected and assayed for their PK-C (Ca^{2+}, phospholipid stimulated), protamine kinase and casein kinase activities.

3. RESULTS AND DISCUSSION

The specific activities of several protein kinases in normal and transformed thymocytes are reported in fig.1. Fig.1A refers to the cytosolic fraction and shows that while cAMP-dependent protein kinase and casein kinase-1 (heparin insensitive) are virtually unchanged in tumoral cells, casein kinase-2 (heparin inhibited) is increased in both M-MuLV and A-MuLV transformed cells. Such an increment is not evident in thymocytes which have been stimulated to proliferate with concanavalin A (netted bars). Unlike CK-2, Ca^{2+}, phospholipid-dependent protein kinase-C is almost unchanged in M-MuLV transformed cells while it undergoes a remarkable increment in the cytosolic fraction of A-MuLV transformed thymocytes. The same is true of tyrosine protein kinase activity whose appearance in A-MuLV transformed cells is seemingly attributable, at least in part, to the kinase expressed by the *v-abl*-oncogene.

A parallel scrutiny of the NP40 extract of the particulate fraction (fig.1B) discloses a situation similar to that of the cytosol: in this case however

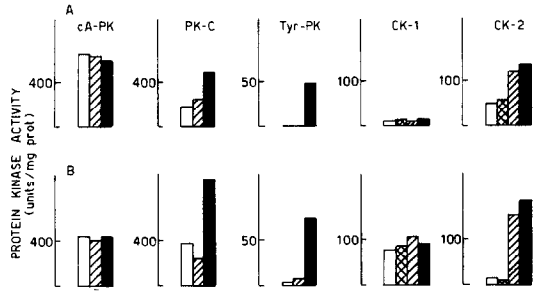

Fig.1. Evaluation of cAMP-dependent protein kinase (cA-PK), protein kinase-C (PK-C), tyrosine protein kinase, casein kinase-1 (CK-1) and casein kinase-2 (CK-2) in the cytosol (A) and NP40 extractable particulate fraction (B) of normal thymocytes (open bars) and of cell lines transformed by either Moloney (hatched bars) or Abelson leukemia viruses (filled bars). Netted bars, where shown, refer to the activity of thymocytes stimulated with concanavalin A. The results of a typical experiment are reported. Very similar values were obtained with 6 different preparations (variations of specific activities never exceeded 10%).

a direct evaluation of Ca^{2+}, phospholipid-dependent activity resulted impossible, probably due to the presence of detergent, and PK-C was evidenced using protamine, a rather specific substrate whose phosphorylation does not require addition of activators [14]. By such an approach protamine kinase activity, as well as tyrosine protein kinase activity, were found substantially increased only in the membranes of cells acutely transformed by A-MuLV. On the other hand, casein kinase-2, almost absent in the particulate fraction of normal cells either quiescent or activated by concanavalin-A, reaches notable levels in the membranes of cells infected with either M-MuLV or A-MuLV. cAMP-dependent protein kinase and CK-1 do not undergo any remarkable variations. The reproducibility of these data has been checked by comparing normal and transformed cells of six different preparations: the increments of CK-2, PK-C and tyrosine protein kinase outlined in fig.1 were constantly observed in all preparations.

To make sure that the heparin inhibited casein kinase activity detected in the membranes of transformed cells is actually due to casein kinase-2, its chromatographic behaviour on DEAE-Sepharose and its responsiveness to specific effectors were checked: most of the casein kinase activity exhibited retarded elution from DEAE-Sepharose, as expected for CK-2 (fig.2A). Furthermore by all the criteria listed in table 1 (stimulation by polylysine and spermine, inhibition by polyglutamate, capability of utilizing GTP) such a retarded casein kinase peak was attributable to CK-2 rather than to CK-1 or to other forms of casein kinase.

On the other hand, the increased protamine kinase activity of A-MuLV transformed cell membranes was almost entirely due to PK-C as also proven by DEAE-Sepharose chromatography (fig.2B) showing that over 80% of it is eluted as a Ca^{2+}, phospholipid-dependent peak, while the remaining activity is more retarded, as expected for the spontaneously active proteolytic derivative of PK-C (PK-M).

A very similar increment of PK-C has also been observed in a cell line obtained by transforming with A-MuLV pre-B lymphocytes, rather than thymocytes (fig.3): also in this case such an increment is accompanied by the appearance of cytosolic tyrosine protein kinase and by an increase of CK-2, whereas cAMP-dependent protein kinase and CK-1 are not significantly altered.

In conclusion our data disclose a remarkable increment of PK-C activity in both soluble and particulate fractions of lymphoid cells which have been transformed by Abelson leukemia virus whose oncogene codes for a tyrosine protein kinase. This observation is especially important in the light of previous reports showing that A-MuLV infection also induces increased Ser/Thr protein phosphorylation in the transformed cells, affecting in particular the ribosomal protein S6 [15], whose

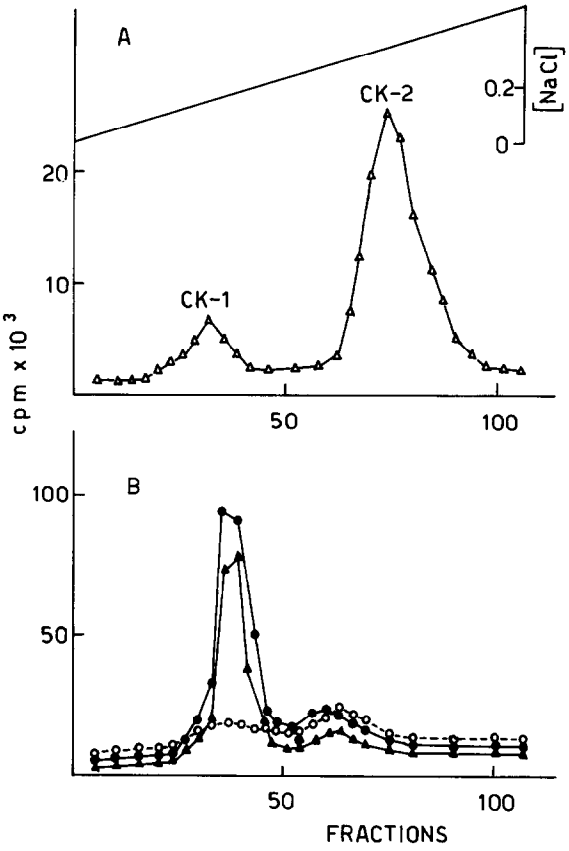

Fig.2. Resolution by DEAE-Sepharose gradient chromatography of casein kinase (A) and protein kinase-C (B) activities extracted with NP40 from the particulate fraction of TA-2 cells transformed by Abelson leukemia virus. (Δ—Δ) Casein kinase; (▲—▲) protamine kinase; (●—●) and (○---○) activities toward the synthetic peptide Orn_4-Tyr-Gly-Ser-Orn_6-Tyr in the presence and absence of Ca^{2+} (500 μM) and phosphatidylserine (50 μg/ml), respectively.

Table 1

Influence of various effectors on casein kinase-2 isolated from the particulate fraction of TA-2 cells transformed by Abelson leukemia virus

Additions	Casein kinase activity
None	100
Heparin (10 μg/ml)	17
Polyglutamic acid (0.2 mg/ml)	43
Unlabeled GTP (100 μM)	46
Polylysine (0.2 mg/ml)	420
Spermine (0.4 mM)	344

Extraction of casein kinase activity and DEAE-Sepharose column chromatography were performed as described in section 2. The fractions of the more retarded predominant peak (see also fig.2A) were pooled and casein kinase activity assayed in the presence of various effectors. Activities are expressed as percent of control

susceptibility to PK-C has recently been demonstrated [16,17]. Considering that no appreciable increment of PK-C activity could be observed in cells transformed by M-MuLV, whose genome lacks any oncogene coding for tyrosine protein kinase, it is tempting to speculate that the rise of PK-C in A-MuLV transformed cells is directly or indirectly triggered by the tyrosine protein kinase expressed by *v-abl*-oncogene, through a cascade mechanism.

On the other hand, a different mechanism must be responsible for the high CK-2 activity which is observed in transformed lymphocytes infected by either A-MuLV or M-MuLV as opposed to quiescent and proliferating normal cells. Seemingly such a rise in CK-2 activity is a common feature of transformed cells and appears to be in good agreement with a previous report indicating a rise of CK-2 as a general property of cell lines which have undergone immortalization [18]. Our additional finding that such an increment of CK-2 in lymphoid cells is largely accounted for by the de novo appearance of a membrane bound form of this enzyme provides a new hint for insight into the possible relationships between alterations of CK-2 and carcinogenesis.

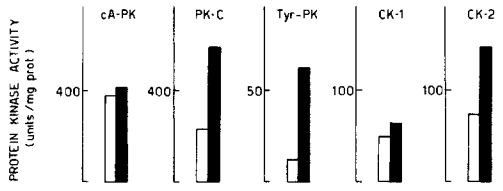

Fig.3. Evaluation of different protein kinase activities in the cytosol of B-lymphocytes (open bars) and of a pre-B cell line transformed by Abelson leukemia virus (filled bars). Symbols are the same as in fig.1. B-lymphocytes were obtained by stimulating mouse spleen cells suspension with LPS. The results are the mean of 4 experiments (variations never exceeded 8%).

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