High tyrosine protein kinase activities in soluble and particulate fractions in bone marrow cells

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High tyrosine protein kinase activities were detected in soluble and particulate forms from bone marrow cells using synthetic peptide (Glu-Asp-Ala-Glu-Tyr-Ala-Ala-Arg-Arg-Arg-Gly) as a substrate. Total activity of tyrosine protein kinase was 2.4 times higher in the soluble fraction but the specific activity was slightly higher in the particulate one. Mg^{2+} or Mn^{2+} requirements of these two enzymes for maximal activity were quite different from each other. Physiological significance of these two forms of enzymes is briefly discussed.

Protein phosphorylation *Tyrosine protein kinase* *Bone marrow* *Proliferation* *Differentiation*

1. INTRODUCTION

Protein phosphorylation on tyrosine residue has been shown to be associated with cell transformation induced by various viruses [1–3], cell growth and multiplication with the action of certain hormones such as epidermal growth factor (EGF) [4], platelet-derived growth factor (PDGF) [5], and insulin [6]. Recently it was discovered that normal cells showed very high tyrosine protein kinase activities in erythrocytes [7], platelets [8] and spleen [9]. In these cells or tissues closely relating to blood function, almost all tyrosine protein kinase activities, if not all, are associated with membranes. The *src* gene product, originally in cytosol, has been considered to move to the plasma membranes via cell transformation [10]. It is therefore very interesting to see whether tyrosine protein kinase exists originally in cytosol of bone marrow cells which produce blood cells in the body. In the present studies we found high tyrosine protein kinase activities in the soluble and the particulate fractions in bone marrow cells and these enzyme properties are briefly studied.

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2. MATERIALS AND METHODS

Homogeneous preparations of rabbit muscle glycogen phosphorylase kinase and cyclic AMP-dependent protein kinase were obtained as in [11] and [12], respectively. A synthetic peptide, $E_{11}G_1$ (Glu-Asp-Ala-Glu-Tyr-Ala-Ala-Arg-Arg-Arg-Gly), was prepared as in [13]. [γ-^{32}P]ATP was prepared as in [14]. Sodium orthovanadate was purchased from Wako Pure Chemicals Industries (Osaka) and Nonidet P-40 from Nakarai Chemicals (Kyoto). All other chemicals of highest grade were obtained from commercial sources. Tyrosine protein kinase activity was measured using a synthetic peptide as a substrate, as in [9]. Protein concentration was determined as in [15]. Phosphoamino acids were analyzed as in [19]. All experiments were carried out with at least three preparations and the assays were carried out in duplicate.

3. RESULTS

3.1. *Enzyme preparations*

Bone marrow clumps were removed from femur and tibia bones of 10 male Sprague-Dawley rats

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and suspended in a small volume of 0.25 M sucrose solution. The clumps were transferred to a 10-ml syringe and quite vigorously expelled out into a beaker cooled in ice through two layers of cheesecloth to create a single-cell suspension. Cells were washed twice by centrifugation for 5 min at 1000 × *g*. The final pellet was resuspended in 3 ml buffer A (50 mM Tris–HCl at pH 7.5, 2 mM $MgCl_2$ and 1 mM EDTA). The cells were sonicated for 30 s using Tomy Seiko Ultrasonic Disrupter, Model UR-200P setting the output dial at 6.5. The sonicate was centrifuged at 1000 × *g* for 10 min to remove nuclear fraction and unbroken cells. The resultant supernatant was then centrifuged at 99000 × *g* for 60 min to prepare soluble and particulate fractions. The particulate fraction was suspended in a small volume of buffer A by homogenization. These preparations thus far obtained were subjected to the soluble or the particulate enzyme in the present studies.

3.2. *Use of synthetic peptide $E_{11}G_1$ as a probe of tyrosine protein kinase activity in bone marrow preparations*

For tyrosine protein kinase assay in a lymphoma cell line, LSTRA [16], the EGF receptor protein kinase [17,18] and the normal tissues [9], the validity of using synthetic peptide having amino acid sequences similar to the tyrosine phosphorylation site of $pp60^{src}$ is widely recognized. As shown in fig.1, in the presence of $E_{11}G_1$ the time course showed almost the linearity for 10 min but in the absence of $E_{11}G_1$ no radioactivity was detected. Phosphoamino acid analysis showed that tyrosine was the only residue being phosphorylated (not shown). Cyclic AMP-dependent protein kinase or glycogen phosphorylase kinase did not phosphorylate $E_{11}G_1$ (not shown). These results show that the assay system employed here is specific for tyrosine protein kinase activity in bone marrow preparations.

3.3. *Intracellular distribution of tyrosine protein kinase in bone marrow and comparison between soluble and particulate enzymes*

Table 1 shows the typical result of intracellular distribution of tyrosine protein kinase. Bone marrow supernatant has 2.4 times higher tyrosine protein kinase activity than the particulate fraction but conversely the specific activity is slightly higher

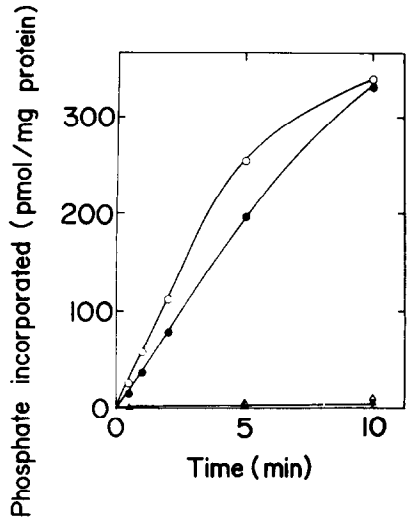

Fig.1. Time course of $E_{11}G_1$ phosphorylation by the soluble and the particulate enzymes in bone marrow cells. Activities of the soluble enzyme (●, ▲) or the particulate one (○, △) were measured in the presence (●, ○) or in the absence (▲, △) of $E_{11}G_1$. Tyrosine protein kinase activity was measured by estimating the phosphorylation of $E_{11}G_1$ (1.0 mM) using 60 μM [γ-^{32}P]ATP and 50 mM Mg^{2+}. In the particulate fraction Nonidet P-40 was added to a final concentration of 0.05%. The amount of protein used was 30 μg/tube for both enzymes.

in the particulate fraction. This distribution pattern was further confirmed by using an Ultra-Turrax homogenizer (Ika-Werk, FRG) instead of sonication (not shown). This is particularly noteworthy in that almost all tyrosine protein kinase activities, including *src* kinase [19], EGF

Table 1

Intracellular distribution of tyrosine protein kinase activity in bone marrow cells

Preparation	Volume (ml)	Protein (mg)	Total activity (pmol/min)	Specific activity (pmol·min^{-1}·mg^{-1})
Soluble	2.7	68.3	2691	39.4
Particulate	1	22.5	1134	50.4

Assay conditions were as in fig.1

Table 2

The effect of Nonidet P-40 on the soluble and the particulate enzymes in bone marrow cells

Preparation	Tyrosine protein kinase activity ($pmol \cdot min^{-1} \cdot mg^{-1}$)	
	− NP40	+ NP40
Soluble	39.4	36.5
Particulate	7.3	50.4

Tyrosine protein kinase activity was measured with or without 0.05% Nonidet P-40 (NP40). Other conditions were as in fig.1

receptor protein kinase [4], PDGF receptor protein kinase [20] and insulin receptor protein kinase [6], are associated with cell membranes or particulate fractions. It is therefore quite interesting to know the differences between the soluble and the particulate enzymes. Table 2 shows the effect of Nonidet P-40, a nonionic detergent, on the soluble and the particulate enzymes. Bone marrow particulate enzyme was activated about 7-fold by 0.1% Nonidet P-40, whereas the soluble enzyme was not (table 2). This activation by Nonidet P-40 seems to be a general characteristic of membrane-bound enzymes, as seen in [9].

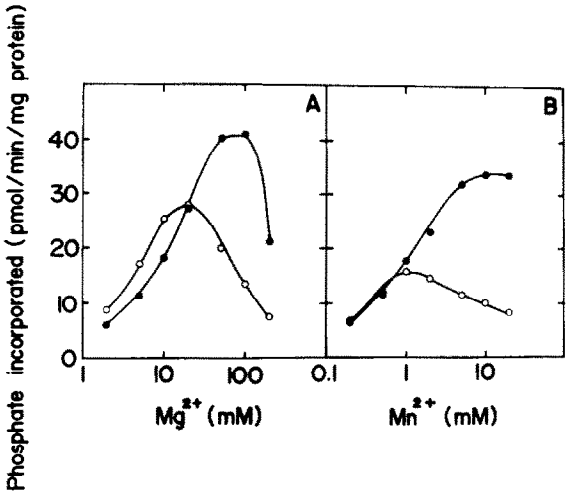

Fig.2. Effects of Mg^{2+} and Mn^{2+} on the soluble and the particulate enzymes in bone marrow cells. Tyrosine protein kinase activity (soluble (●), particulate (○)) was measured as a function of $[Mg^{2+}]$ (A) or $[Mn^{2+}]$ (B). Assay conditions were as in fig.1.

For further characterization of the soluble and the particulate enzymes, the effects of Mg^{2+} and Mn^{2+} were tested (fig.2). Both Mg^{2+} and Mn^{2+} could serve as metal ions for the phosphorylation of $E_{11}G_1$. The soluble enzyme required 50–100 mM Mg^{2+} concentration for maximal activity, whereas the particulate enzyme showed 10–20 mM (fig.2A). Further we observed that for the degree of requirements for Mn^{2+} both enzymes showed similar dissociation patterns as obtained with Mg^{2+} dependency (fig.2B). And the particulate enzyme had a several times higher K_m value for ATP than the soluble one (not shown). These results strongly suggest that different kinds of tyrosine protein kinases may exist in bone marrow cytosol and membrane.

4. DISCUSSION

In the present studies relatively high tyrosine protein kinase activities were detected in both soluble and particulate fractions. It is not clear at present whether these two enzymes are of the same origin or not. If they are of the same origin, it may be possible that, like the *src* gene product, the soluble enzyme moves to the cell membranes during cell proliferation or differentiation [10], or, like Ca^{2+}-activated, phospholipid-dependent protein kinase (protein kinase C), a certain factor (such as Ca^{2+}) may regulate the distribution of this enzyme between cytosol and cell membrane [21]. If they are of different origins, the soluble enzyme should be a novel type of tyrosine protein kinase not yet reported. Differences of Mg^{2+} or Mn^{2+} requirement for both enzymes (fig.2) may support the latter possibilities.

Further characterization of these two enzymes is necessary to reveal their physiological roles in cell proliferation and differentiation.

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