

A protein-tyrosine kinase in the nuclear matrix from rat liver

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Protein kinase activity in isolated nuclei from rat liver was detected *in situ* after electrophoresis on SDS-polyacrylamide gel containing no exogenous protein substrate. After renaturation of polypeptides, the gel was incubated with [γ - 32 P]ATP and divalent cations. Among five major protein kinase activities observed as radioactive bands by autoradiography, a protein kinase autophosphorylating on tyrosine (M_r 30 000) was identified and found to be localized in the nucleus, particularly in the nuclear matrix. The intensity of the activity band representing the level of the protein-tyrosine kinase in rat liver nuclei did not appreciably change during 3–24 h after partial hepatectomy.

Protein-tyrosine kinase Autophosphorylation Nuclear matrix (Rat liver)

1. INTRODUCTION

Protein-tyrosine kinases seem to play important roles in the regulation of cellular growth and development [1,2]. All of the protein-tyrosine kinases found to date are located in the cytoplasm [2], and little attention has been focused on nuclear protein-tyrosine phosphorylation. We have applied an activity gel method (*in situ* assay of enzyme after SDS-polyacrylamide gel electrophoresis, SDS-PAGE) to detect protein-tyrosine kinases that are presumed to be present in nucleus. This method has been successfully employed for DNA-metabolizing enzymes and some other enzymes using macromolecular substrates [3–7]. Here we report the existence of a nuclear protein kinase capable of autophosphorylating on tyrosine, which is associated with the nuclear matrix.

2. MATERIALS AND METHODS

2.1. Materials

[γ - 32 P]ATP (3000 Ci/mmol) was purchased from ICN; cellulose-coated thin-layer plates from Eastman-Kodak; DNase I from Takara; phosphoserine and phosphothreonine from Sigma; trypsin from Boehringer. Phosphotyrosine was synthesized as in [8].

2.2. Subcellular fractionation of rat liver

All operations were carried out at 4°C. Fresh livers (2–3 g) from normal and 70%-hepatectomized Wistar male rats weighing 100–200 g were homogenized in 5 vols of 0.25 M sucrose/3.3 mM MgCl₂ in a glass-Teflon homogenizer, and the homogenates were centrifuged at 1000 × *g* for 10 min. The resulting pellets were resuspended in 15 ml of 2.2 M sucrose/3.3 mM MgCl₂ and then gently layered on a 10 ml cushion of 2.2 M sucrose/3.3 mM MgCl₂. The nuclei were precipitated by centrifugation at 40 000 × *g* for

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60 min, followed by washing twice with 0.25 M sucrose/3.3 mM MgCl_2 . Isolation of the nuclear matrix was carried out by a modification of the method of Berezney and Coffey [9]. The nuclear pellet was suspended in 2 M NaCl/10 mM Tris-HCl, pH 7.5/10 mM MgCl_2 /1 mM phenylmethylsulfonyl fluoride and digested with DNase I (100 $\mu\text{g}/\text{ml}$) at 37°C for 60 min. The resulting nuclear matrix was collected by centrifugation at $1500 \times g$ for 15 min and washed with 2 M NaCl/10 mM Tris-HCl, pH 7.5/0.1% Triton X-100/1 mM EDTA/1 mM phenylmethylsulfonyl fluoride. Plasma membrane and mitochondria [10] and post-nuclear particular fraction [11] were prepared from rat liver homogenate. Protein was determined by the method of Bradford [12].

2.3. *In situ* assay of protein kinase after SDS-PAGE (activity gel method)

Polyacrylamide gel electrophoresis in the presence of SDS was carried out as in [7]. The samples were directly dissolved in 62.5 mM Tris-HCl, pH 6.8/2% SDS/10% glycerol/5% 2-mercaptoethanol (sample buffer), incubated at 40°C for 10 min, and then immediately loaded onto an SDS-10% polyacrylamide slab gel (1 mm depth) containing no exogenous protein substrate with a 3% stacking gel. After electrophoresis, the gel was washed three times for 30 min each with 300 ml of 50 mM Tris-HCl, pH 7.5, at room temperature with mild agitation, followed by storage in 500 ml of 50 mM Tris-HCl, pH 7.5/0.5 mM dithiothreitol at 4°C for 20–24 h. The gel was then incubated at 30°C for 15 h in a reaction mixture (10 or 20 ml) containing 50 mM Tris-HCl (pH 7.5), 5 mM MgCl_2 , 5 mM MnCl_2 , 0.5 mM dithiothreitol and 5 nM $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ (100 $\mu\text{Ci}/10\text{ ml}$) with mild agitation. Autoradiography of the gel was carried out after washing with 5% trichloroacetic acid/1% NaPP_i [7].

2.4. Analysis of phosphoamino acids

The radiolabeled bands were excised from the gels and digested at 37°C for 1 h in 300 μl of 50 mM $(\text{NH}_4)_2\text{CO}_3$ containing 10 μg trypsin. After lyophilization, samples were hydrolyzed with 6 M HCl at 110°C for 1 h. The hydrolysates were lyophilized, dissolved in H_2O and subjected to electrophoresis on cellulose thin-layer plates at pH 3.5 for 1 h at 400 V [13]. Autoradiography was

carried out at -70°C for 48 h. Authentic phosphoserine, phosphothreonine and phosphotyrosine were detected by ninhydrin staining.

3. RESULTS AND DISCUSSION

We applied the activity gel method to the detection of nuclear protein kinase activity. A nuclear pellet from rat liver was directly separated into protein components by electrophoresis on an SDS-polyacrylamide gel containing no exogenous protein. Following removal of SDS, the gel was incubated in a reaction mixture containing $[\gamma\text{-}^{32}\text{P}]\text{ATP}$, MgCl_2 , and MnCl_2 to allow the phosphotransferase reaction to occur. Among radiolabeled bands representing phosphorylated

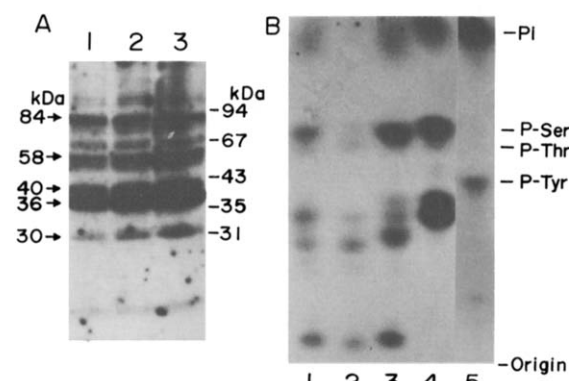


Fig.1. *In situ* detection of protein kinases in nuclei of rat liver after SDS-PAGE. (A) Total nuclear proteins were analyzed by SDS-PAGE. The activity gel method was carried out as described in section 2. Lane 1, 30 μg protein; lane 2, 60 μg protein; lane 3, 120 μg protein. Numbers on the right refer to the molecular masses of marker polypeptides: phosphorylase *b* (94 kDa); bovine serum albumin (67 kDa); ovalbumin (43 kDa); lactate dehydrogenase (35 kDa); carbonic anhydrase (31 kDa). The migration positions of the 84-, 58-, 40-, 36- and 30-kDa protein are indicated. (B) Phosphoamino acids of the phosphorylated protein bands in (A) were separated by electrophoresis on cellulose thin-layer plates, detected by autoradiography and identified by co-migration with authentic phosphoserine (P-Ser), phosphothreonine (P-Thr) and phosphotyrosine (P-Tyr). Lane 1, 84-kDa protein; lane 2, 58-kDa protein; lane 3, 40-kDa protein; lane 4, 36-kDa protein; lane 5, 30-kDa protein.

proteins, five major and reproducible bands corresponding to 84, 58, 40, 36 and 30 kDa were observed (fig.1A). To identify the phosphoamino acids present in these proteins, each protein band was eluted from the polyacrylamide gel by digestion with trypsin and acid-hydrolyzed. Following electrophoretic separation of phosphoamino acids, the 30-kDa protein was found to be phosphorylated exclusively at tyrosine residues, the other four proteins being phosphorylated at serine (84-, 40- and 36-kDa protein) or threonine residues (58-kDa protein) (fig.1B). Since the polyacrylamide gel included no exogenous protein substrate, it seems likely that tyrosine phosphorylation of the 30-kDa protein represents an autophosphorylation reaction. The 30-kDa protein seems to be present *in vivo* as a monomeric enzyme or an active subunit of the oligomeric enzyme, because the freshly prepared nuclear pellet was directly treated with sample buffer containing SDS, followed by SDS-PAGE.

We next examined the subcellular localization of the 30-kDa protein kinase. The 30-kDa phosphoprotein was observed in nuclei of rat liver, but not in cytoplasmic fractions such as the post-nuclear particulate fraction, plasma membrane

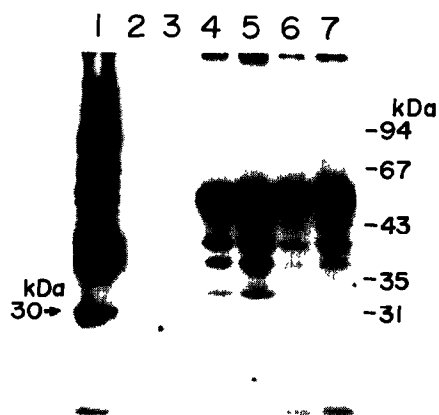


Fig.2. Subcellular localization of protein-tyrosine kinase (30 kDa). Proteins from rat liver nuclei (lane 1, 80 µg), particulate fraction (lane 2, 40 µg; lane 3, 80 µg), plasma membrane (lane 4, 40 µg; lane 5, 80 µg) and mitochondria (lane 6, 40 µg; lane 7, 80 µg) were analyzed by SDS-PAGE. The activity gel method was carried out as described in section 2. Positions of the 30-kDa protein and marker polypeptides (see fig.1A) are indicated.

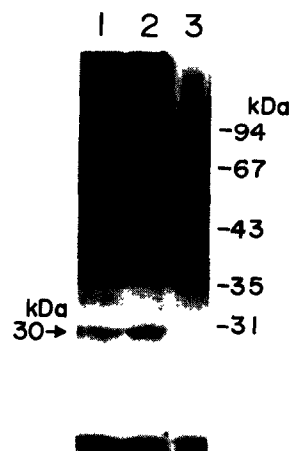


Fig.3. Subnuclear localization of protein-tyrosine kinase (30 kDa). Nuclear matrix and 2 M NaCl extract from rat liver nuclei were analyzed by SDS-PAGE. The activity gel method was carried out as described in section 2. Lane 1, nuclei (180 µg protein); lane 2, nuclear matrix (300 µg protein); lane 3, 2 M NaCl extract (190 µg protein). Positions of the 30-kDa protein and marker polypeptides (see fig.1A) are indicated.

and mitochondria (fig.2). Furthermore, the 30-kDa protein was found to be localized exclusively in the nuclear matrix (fig.3). Recently, a protein-tyrosine kinase of 30 kDa has been detected in post-nuclear particulate fraction of lymphocytes by the activity gel method [14]. The relationship between our 30-kDa protein kinase and the lymphocyte tyrosine kinase remains obscure.

Since protein-tyrosine kinases have been found in cytoplasmic fractions (membrane fraction, cytoskeleton, cytosol, etc.) [2], this is the first report of a protein-tyrosine kinase localized in the nucleus, particularly in the nuclear matrix. Tyrosine phosphorylation seems to be involved in cellular growth control [1,2]. In addition, the nuclear matrix is suggested to be involved in DNA replication, transcription and post-transcriptional regulation [15–17]. Therefore, it is tempting to speculate that this protein-tyrosine kinase may play an important role in cellular proliferation. Partial hepatectomy of rat was carried out to determine whether the activity level of the protein-tyrosine kinase fluctuates after the operation. We observed no appreciable change in intensity of the

activity bands representing the level of the kinase in nuclei and the nuclear matrix of rat liver during 3–24 h after the operation (not shown). Further work is required to reveal a physiological role of the protein-tyrosine kinase.

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