Glycogen synthase (casein) kinase-1: tissue distribution and subcellular localization

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The distribution of glycogen synthase (casein) kinase-1 (CK-1) among different rat tissues and subcellular fractions was investigated. Using casein, glycogen synthase and phosphorylase kinase as substrates, CK-1 activity was detected in kidney, spleen, liver, testis, lung, brain, heart, skeletal muscle and adipose tissue. The distribution of CK-1 among different subcellular fractions of rat liver was; cytosol (72.1%), microsome (17.6%), mitochondria (9.6%) and nuclei (0.7%). CK-1 from rat tissues was shown to have a similarly wide substrate specificity as highly purified CK-1 from rabbit skeletal muscle. Such wide substrate specificity and distribution among different mammalian tissues and subcellular organelles indicate that CK-1 may be involved in the regulation of diverse cellular functions.

Phosphorylation *Casein kinase* *Multifunctional enzyme* *Widespread distribution*

1. INTRODUCTION

Glycogen synthase (casein) kinase-1 (CK-1) is a cyclic nucleotide- and calcium-independent protein kinase first identified in rabbit skeletal muscle [1] and later in rat and rabbit liver [2,3]. Skeletal muscle CK-1 was initially identified as a glycogen synthase kinase that could also phosphorylate casein and phosvitin. However, we have since shown that CK-1 has a very wide substrate specificity and may therefore be involved in the regulation of diverse cellular functions [4].

In this study we provide further evidence that agrees with the above hypothesis. We found that CK-1 is widely distributed among different rat tissues and among the different subcellular fractions of a particular tissue.

Abbreviations: TPCK, L-1-tosylamido-2-phenylethyl chloromethyl ketone; TLCK, *N*-α-*p*-tosyl-L-lysine chloromethyl ketone; PMSF, phenylmethylsulfonyl fluoride

2. EXPERIMENTAL

2.1. *Materials*

Tubulin [5] and microtubule-associated protein-2 [6] were purified from rat brain. Myosin light chain [7] was purified from turkey gizzard and was a mixture of the 20 and 17 kDa light chains. Glycogen synthase [8] and phosphorylase kinase [9] were purified from rabbit skeletal muscle. [γ-^{32}P]ATP was from New England Nuclear. Tissues from Sprague-Dawley rats (150–200 g) were rapidly removed after killing, frozen in liquid nitrogen and stored at −70°C.

2.2. *Tissue distribution of CK-1*

Frozen rat tissues (12–15 g) were thawed, cut into small pieces and homogenized for 30 s, twice in 3 vols of Buffer A [50 mM Tris-HCl (pH 7.5) containing 1 mM dithiothreitol, 1 mM EDTA, 0.25 M sucrose, 0.5 mM PMSF, 0.05 mM TPCK, 0.05 mM TLCK, and 1 mg/l each of pepstatin A, leupeptin and chymostatin]. The homogenate was centrifuged at 10000×*g* for 15 min, then at

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100 000 × *g* for 1 h. The supernatant was applied to a phosphocellulose column (1.5 × 7 cm) equilibrated with Buffer A + 0.1 M KCl. The column was washed with 25 ml Buffer A + 0.10 M KCl followed by 50 ml Buffer A + 0.35 M KCl. CK-1 and CK-2 were eluted consecutively from the column with 25 ml Buffer A + 0.60 M KCl and 25 ml Buffer A + 1.0 M KCl, respectively. The eluted enzymes were diluted with Buffer A to reduce KCl concentration to 0.3 M and concentrated to 1 ml by ultrafiltration.

2.3. *Subcellular localization of CK-1 in rat liver*

Cytoplasmic, mitochondrial and microsomal fractions were prepared as described [10]. The liver (15 g) from a freshly killed rat was homogenized (12 strokes) in 3 vols of Buffer A utilizing a Potter-Elvehjem homogenizer with a loose fitting Teflon pestle. The homogenate was filtered through 4 layers of cheese cloth and centrifuged at 600 × *g* for 10 min. The pellet was discarded and the supernatant was centrifuged at 11 500 × *g* for 10 min. The pellet containing mitochondria was washed twice with Buffer A and resuspended in the same buffer. The postmitochondrial supernatant was centrifuged at 100 000 × *g* for 60 min. The microsomal pellet was washed twice with Buffer A and resuspended in the same buffer. The supernatant from the initial centrifugation at 100 000 × *g* was used as the cytosol fraction.

Nuclei were separately prepared from a fresh liver (15 g) as described [11]. Briefly, the liver was rinsed in Buffer B [50 mM Tris-HCl (pH 7.5) containing 25 mM KCl, 5 mM $MgCl_2$, and 1 mM PMSF] containing 0.25 M sucrose. The liver was then homogenized in 2 vols of Buffer B containing 0.25 M sucrose as described above. To each 3 ml portion of homogenate was added 6 ml of Buffer B containing 2.3 M sucrose. After mixing, the solution was underlaid with 3 ml of Buffer B containing 2.3 M sucrose. The solutions were then centrifuged at 124 000 × *g* for 30 min at 3°C in a SW 41 rotor. The supernatant was discarded and the pellets containing nuclei were resuspended in Buffer A.

CK-1 and CK-2 were solubilized from mitochondrial, microsomal and nuclear fractions by the addition of sodium deoxycholate and KCl to final concentrations of 0.3% and 1.0 M, respectively. After sonification (3 times, each for 10 s with an interval of 10 s) at maximum power of a Kontes Sonifier the fractions were gently stirred at 4°C for 30 min. They were then centrifuged at 100 000 × *g* for 1 h. The supernatant was used to purify CK-1 and CK-2 on a phosphocellulose column as outlined above.

2.4. *Enzyme assays*

CK-1 and CK-2 were assayed at 30°C in a reaction mixture (0.05 ml) containing 4 mg/ml casein, 25 mM Tris-HCl buffer (pH 7.4), 1 mM dithiothreitol, 0.5 mM EGTA, 5 mM KF, 10 mM magnesium acetate, 0.10 mM [γ-^{32}P]ATP. The reactions were initiated by the addition of [γ-^{32}P]ATP and aliquots were removed at different times for the determination of ^{32}P incorporation into protein [12]. One unit of Ck-1 or CK-2 is defined as the amount of enzyme catalyzing the incorporation of 1 pmol ^{32}P from [γ-^{32}P]ATP into casein (4 mg/ml) per min at 30°C. Lactate dehydrogenase [13], glutamate dehydrogenase [14] and glucose-6-phosphatase [15] were assayed as described.

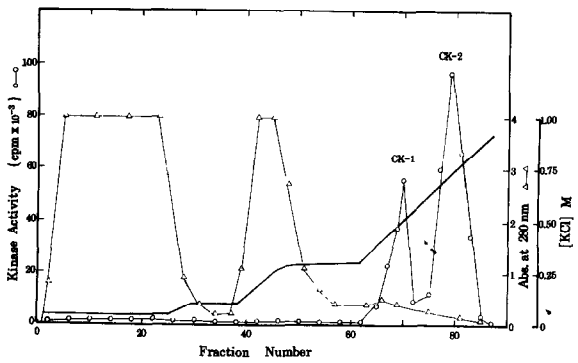

Fig.1. Fractionation of rat liver cytosol by phosphocellulose chromatography. The soluble fraction (100 000 × *g* supernatant) from rat liver (34 g) was prepared and applied to a phosphocellulose column (2 × 11 cm) equilibrated with Buffer A. The column was washed with Buffer A + 0.10 M KCl followed by Buffer A + 0.35 M KCl. The column was finally eluted with a linear gradient of KCl (0.35 → 1.2 M). Fractions of 6 ml were collected. Aliquots (0.005 ml) were used for measuring casein kinase activity (○). (△) Absorbance at 280 nm; (——) KCl concentrations.

3. RESULTS

3.1. *Separation of CK-1 and CK-2 by phosphocellulose chromatography*

When the soluble fraction from rat liver is fractionated on a phosphocellulose column two casein kinase activity peaks can clearly be resolved (fig.1), as also shown by others [2]. These kinase eluted at approx. 0.5 and 0.7 M KCl and have been termed casein kinase-1 (CK-1) and casein kinase-2 (CK-2), respectively. Our previous studies have shown that CK-1 can phosphorylate many other substrates in addition to casein [4]. From fig.1 it can also be seen that CK-1 represents only about one-third of the total casein kinase activity. Further, we have determined that cyclic AMP-dependent protein kinase, calcium- and phospholipid-dependent protein kinase [16], and kinase F_A [17] either do not bind to the phosphocellulose column or are eluted at lower KCl concentrations than do CK-1 and CK-2 (not shown). This observation is important because our results, especially on the substrate specificity of CK-1 (see fig.2), cannot be explained as being due to the action of these enzymes.

3.2. *Wide distribution of CK-1 among different rat tissues*

We have surveyed for the presence of CK-1 (and CK-2) in the soluble fraction of different rat tissues using the protocol outlined in fig.1. CK-1 was first eluted from the column with 0.6 M KCl; by increasing KCl to 1.0 M CK-2 was eluted. In this way CK-1 and Ck-2 from 9 rat tissues were quantitated (table 1). It can be seen that these enzymes are present in all tissues surveyed, with the highest activities of CK-1 occurring in kidney, spleen, and liver. Similar to the results observed for liver (fig.1) the activity of CK-2 was much higher than CK-1 in all tissues.

3.3. *Phosphorylation of multiple substrates by CK-1*

We have highly purified CK-1 from rabbit skeletal muscle [1] and shown that this enzyme can phosphorylate several other substrates besides casein [4]. However, casein kinases similar to CK-1 and apparently having a more restricted substrate specificity have been described by others [18]. It was therefore essential to demonstrate that CK-1 purified from the different rat tissues (table 1) does

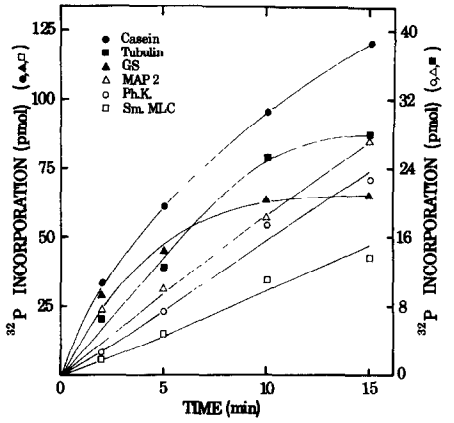

Fig.2. Phosphorylation of multiple substrates by rat kidney CK-1. The different substrates (each at 0.5 mg/ml, except glycogen synthase which was at 0.25 mg/ml) were phosphorylated by rat kidney CK-1 (12 μg) in a reaction mixture (0.05 ml) similar to that described in section 2. Aliquots (0.01 ml) were removed at the different times for the determination of ^{32}P incorporation into protein. Rat kidney CK-1 was partially purified on a phosphocellulose column as outlined in fig.1. (●—●) Casein, (□—□) tubulin, (▲—▲) glycogen synthase [GS], (△—△) microtubule-associated protein-2 [MAP-2], (○—○) phosphorylase kinase [Ph.K.], (□—□) smooth muscle myosin light chain [Sm.MLC].

Table 1

Activities of CK-1 and CK-2 purified from the soluble fraction of different rat tissues

Tissue	Kinase activity (units/g tissue)			Percent
	CK-1	CK-2	Total	CK-1
Kidney	400	460	860	47
Spleen	345	774	1119	31
Liver	232	474	706	33
Testis	157	717	874	18
Lung	133	354	487	27
Brain	125	291	416	30
Heart	116	416	532	22
Skeletal muscle	27	34	61	44
Adipose tissue	4	10	14	29

CK-1 and CK-2 were purified from the soluble fraction of the different tissues as outlined in section 2. The activities of CK-1 and CK-2 were assayed using casein as a substrate. Since CK-2 uses GTP as a substrate and is inhibited by heparin [18], assays for CK-1 contained 0.2 mM GTP and 2 μg/ml heparin to inhibit any contaminating CK-2

Table 2
Subcellular distribution of rat liver CK-1 and CK-2

Cell fraction	Protein (mg/g liver)	Casein kinases						Marker enzymes		
		Activity (units/g liver)		Specific activity (units/mg protein)		Distribution (percent)		LDH (μmol/min per mg protein)	GDH (nmol/min per mg protein)	G-6-Pase
		CK-1	CK-2	CK-1	CK-2	CK-1	CK-2			
Nuclei	0.5	2.2	20.9	4.4	41.8	0.7	3.9	1.6	8.2	1.8
Mitochondria	15.3	30.7	10.9	2.0	0.7	9.6	2.1	1.0	69.9	34.6
Microsomes	15.4	56.4	25.6	3.7	1.7	17.6	4.8	0.7	1.3	283.8
Cytosol	62.9	231.7	473.7	3.7	7.5	72.1	89.2	21.4	5.7	3.9

CK-1 and CK-2 were solubilized from the particulate fractions by treating with 1.0 KCl and 0.3% sodium deoxycholate for 30 min at 4°C. Solubilized CK-1 and CK-2 were then separated on a phosphocellulose column. The activities of these enzymes were assayed using casein as a substrate. The activities of the marker enzymes were assayed after solubilization of the particulate fractions. LDH, lactate dehydrogenase; GDH, glutamate dehydrogenase; G-6-Pase, glucose-6-phosphatase

have a substrate specificity as wide as the rabbit skeletal muscle enzyme. As shown in fig.2, rat kidney Ck-1 can phosphorylate tubulin, glycogen synthase, microtubule-associated protein-2, phosphorylase kinase, smooth muscle myosin light chain and casein. Troponin is also a substrate for the kinase (not shown). All these proteins were previously shown to be substrates for rabbit skeletal muscle CK-1 [4,19]. We did not repeat the experiment shown in fig.2 using CK-1 from each of the different tissues listed in table 1. However, we did find that CK-1 from all 9 tissues can phosphorylate phosphorylase kinase and glycogen synthase under conditions identical to those outlined for fig.2 (not shown). These results, therefore, suggest that CK-1 purified from the different rat tissues in this study is the same as the rabbit skeletal muscle enzyme [4].

3.4. *Subcellular distribution of CK-1*

In keeping with the postulated multiple roles for CK-1 [4] we investigated whether this enzyme is present in different cellular compartments. Since we already know that the activity of CK-1 (and Ck-2) is very high in the cytosol (table 1) it is essential to document whether these enzymes are also present in the particulate fractions. Rat liver homogenate was therefore resolved into different subfractions from which CK-1 and CK-2 were solubilized and separated by phosphocellulose column chromatography. Both CK-1 and CK-2 are present in all 4 subfractions (table 2). However, the distribution of these two enzymes among the various subcellular fractions is different. CK-1 is the predominant form in mitochondria and microsomes, whereas CK-2 is greater than CK-1 in nuclei and cytosol. Further, both CK-1 and CK-2 are largely soluble enzymes (72 and 89% of the total recovered activities, respectively). However, CK-1 activity is significant especially in microsomes (18%) and mitochondria (10%). We found that partially purified CK-1 from rat liver microsomes can also phosphorylate the same substrates as does cytosolic CK-1 (fig.2 and data not shown). These results indicate that besides being widely distributed among different tissues, CK-1 is also present in all cell compartments, thus implying its importance in the regulation of different cellular functions.

4. DISCUSSION

Previous studies have shown that many tissues contain at least two major classes of casein kinases, similar to CK-1 and CK-2 (see fig.1). CK-2 is an oligomeric protein (M_r 135 000) containing two types of subunits termed α (M_r 42 000) and β (M_r 27 000). In addition, the enzyme is inhibited by heparin and can use both ATP and GTP as phosphoryl donors. Using these criteria CK-2 purified from different sources by several laboratories seems to represent the same enzyme [18]. CK-1 from the different sources, by contrast, is more difficult to compare. The enzyme is apparently monomeric and has an M_r of 30 000–40 000. It is

not inhibited by heparin and does not use GTP as a substrate [18]. Because of the unavailability of an inhibitor or activator specific for CK-1, it is difficult to classify CK-1 purified by different laboratories as being the same or different kinases. One way by which such classification can be made is to examine the specificity of CK-1 purified from different sources for defined protein substrates. We have compared the specificity of CK-1 from different rat and rabbit tissues for a variety of protein substrates.

Previously, we have shown that CK-1 highly purified from rabbit skeletal muscle could phosphorylate many different substrates: casein, glycogen synthase, phosphorylase kinase, troponin, myosin light chain and light chain kinase from both smooth and skeletal muscle, tubulin and microtubule-associated protein-2 [4,19]. Here we have used casein, glycogen synthase and phosphorylase kinase as substrates to identify CK-1 from 9 different rat tissues. Further detailed analysis showed that CK-1 from rat kidney can phosphorylate the same proteins that are substrates for CK-1 from rabbit skeletal muscle (fig.2). We have taken this analysis one step further and shown that CK-1 purified from rabbit skeletal muscle (and liver) and rat liver can phosphorylate the same sites on glycogen synthase and smooth myosin light chain (not shown). These results, therefore, suggest that the CK-1 that we studied from rabbit skeletal muscle and liver and the different rat tissues represents a single kinase having a wide substrate specificity.

Many of the substrates phosphorylated by CK-1 are also subtrates for cyclic AMP-dependent protein kinase [4,19]. The wide substrate specificity of the latter kinase, its ubiquitous distribution among mammalian tissues, and its importance is regulating multiple cellular processes is well established [20,21]. Similarly, we propose that the wide substrate specificity of CK-1 and its ubiquitous distribution among mammalian tissues and cell organelles indicate that it is also involved in the regulation of diverse cellular functions. The mode by which CK-1 may regulate different cell functions is still obscure. However, we have recently shown that the activity of CK-1 may be regulated through the action of glucagon in isolated hepatocytes [22]. Further studies are clearly required to evaluate the importance of CK-1 in regulating different cellular processes.

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