FUNCTIONAL SIMILARITY OF YEAST AND MAMMALIAN ADENOSINE 3',5'-MONOPHOSPHATE-DEPENDENT PROTEIN KINASES*

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SUMMARY: Although adenosine 3',5'-monophosphate-dependent protein kinase obtained from *Saccharomyces cerevisiae* shows different physical and kinetic properties from those isolated from mammalian tissues, the yeast and mammalian enzymes appear to be functionally identical; the enzymes are equally active in the phosphorylation of muscle glycogen phosphorylase kinase and glycogen synthetase, resulting in the activation and inactivation of the respective enzymes.

Adenosine 3',5'-monophosphate (cyclic AMP)-dependent protein kinases found in many tissues and organs are shown to be dissociated by the cyclic nucleotide each into catalytic unit and regulatory unit (1-4). All catalytic units of mammalian enzymes thus far obtained are indistinguishable from each other in their kinetic and catalytic properties (5). A recent report from this laboratory has described partial purification and properties of a protein kinase isolated from baker's yeast, *Saccharomyces cerevisiae* (6). Although the occurrence of cyclic AMP in this organism has been described repeatedly (7-9), the mode of action as well as the functional specificity of this particular enzyme has remained unexplored. The experiments briefly described here were designed to show that yeast and mammalian enzymes were completely exchangeable in

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the phosphorylation of muscle glycogen phosphorylase kinase and glycogen synthetase. The evidence implies that in this eukaryotic microorganism the protein kinase may play roles in a similar manner to that proposed for higher organisms.

Yeast and rat liver cyclic AMP-dependent protein kinases were purified with calf thymus histone as substrate in routine assays by the method previously described (6,10). Crystalline rabbit skeletal muscle glycogen phosphorylase b was obtained by the method of Fischer and Krebs (11). Rabbit skeletal muscle glycogen phosphorylase kinase and glycogen synthetase were prepared by the method of Cohen (12) and Soderling *et al.* (13), respectively. Other experimental procedures are specified in the legends of a table and figures.

As described in a preceding report (6), yeast and rat liver cyclic AMP-dependent protein kinases showed slightly different properties: molecular weight of holoenzymes, 58,000 and 100,000∿200,000[1/]; molecular weight of catalytic units, 30,000 and 35,000; isoelectric point, pH 7.7 and pH 4.8∿5.2[1/]; optimum pH, pH 7.5 and pH 7.0; optimum Mg^{++}, 5 mM and 3 mM; Km value for ATP, 1.2×10^{-5} M and 5×10^{-6} M; Ka value for cyclic AMP, 2.0×10^{-8} M and $1∿4 \times 10^{-8}$ M[1/], respectively. Nevertheless, yeast enzyme was shown to be functionally identical with mammalian enzymes which were capable of phosphorylating muscle glycogen phosphorylase kinase, glycogen synthetase and many other proteins. When muscle glycogen phosphorylase kinase and glycogen phosphorylase b were incubated with either yeast or liver protein kinase in the presence of ATP, the phosphorylase activity was greatly enhanced as judged by the formation of glucose 1-phosphate during the subsequent incubation with glycogen and radioactive

1/ In most mammalian tissues including rat liver, multiple species of cyclic AMP-dependent protein kinases are distinguished which are composed of a common catalytic unit and different regulatory units. The variation of molecular weight, isoelectric point and Ka value for cyclic AMP is due to the multiplicity of regulatory units (5,10).

Table I

Activation of muscle glycogen phosphorylase kinase by yeast and liver cyclic AMP-dependent protein kinases

The reaction mixture (0.15 ml) initially contained 2.5 μmoles of Tris-maleate at pH 6.5, 0.9 μmole of magnesium acetate, 0.5 μmole of 2-mercaptoethanol, 10 nmoles of ATP, 0.1 nmole of cyclic AMP, 8.5 μg of crystalline muscle glycogen phosphorylase b free of 5'-AMP, 0.6 μg of muscle glycogen phosphorylase kinase and 24 units of either yeast or liver cyclic AMP-dependent protein kinase. One unit of protein kinase activity was defined as that amount of enzyme which incorporated 1 pmole of the terminal phosphate of ATP into an acid-precipitable material per min when calf thymus histone was employed as substrate under the standard assay conditions described previously (6). After incubation for 5 min at 30°C, 0.03 ml of a solution containing 1.0 mg of glycogen and 2.5 μmoles of ^{32}Pi (63,200 cpm/μmole) was added. The mixture was incubated for additional 5 min at 30°C. Pi was precipitated as a triethylamine-phosphomolybdate complex by the method of Sugino and Miyoshi (14) and radioactive glucose 1-phosphate formed was determined by the method described in the previous paper (5). The numbers given in this table are counts per min of phosphate incorporated into glucose 1-phosphate.

Protein kinase	Phosphorylase kinase	Phosphorylase b	^{32}Pi incorporation into glucose 1-phosphate			
			in the presence of ATP		*in the absence of ATP*	
			c-AMP (+)	c-AMP (-)	c-AMP (+)	c-AMP (-)
None	-	-	0	0	0	0
	-	+	172	186	179	176
	+	-	96	113	87	97
	+	+	1,476	1,278	154	193
Yeast	-	-	5	25	31	9
	-	+	95	181	119	132
	+	-	75	85	73	96
	+	+	3,590	2,670	95	83
Liver	-	-	16	32	35	1
	-	+	86	19	79	105
	+	-	89	73	99	111
	+	+	3,592	1,844	86	103

inorganic orthophosphate as shown in Table I. Practically no glucose 1-phosphate was produced in the absence of ATP. Some activity observed in the absence of protein kinase in this experiment appeared to be due to autocatalytic phosphorylation of glycogen phosphorylase kinase as described by Krebs (15), or due to the activated form of

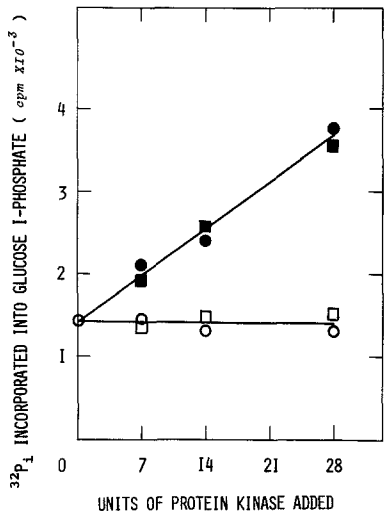

Fig. 1 *Activation of muscle glycogen phosphorylase b kinase by varying concentrations of yeast and rat liver cyclic AMP-dependent protein kinases.* The detailed experimental conditions are identical with those given in Table I except that varying concentrations of either yeast or rat liver cyclic AMP-dependent protein kinase were added and all reactions were carried out in the presence of 0.1 nmole of cyclic AMP. Unit of protein kinase activity is defined in Table I. ●——● and o——o represent experimental values with yeast protein kinase in the presence and absence of ATP, respectively; ■——■ and □——□ with rat liver protein kinase in the presence and absence of ATP, respectively.

phosphorylase kinase which slightly contaminated the preparation. The experiments given in Fig. 1 indicated that the relative efficacy of yeast and liver protein kinases was identical in the phosphorylation reaction. Similar results were also obtained with protein kinases partially purified from other mammalian tissues such as rabbit skeletal muscle and rat brain.

Previous reports from this laboratory (5,16,17) have shown that muscle and liver protein kinases are equally active in the phosphorylation of muscle glycogen synthetase. In the experiment given in Fig. 2, muscle glycogen synthetase was shown to be inhibited progressively by the preincubation with increasing amounts of either yeast or liver protein kinase in a parallel fashion in the presence of ATP. In this experiment catalytic units of the kinases were employed. When holoenzymes of cyclic AMP-dependent

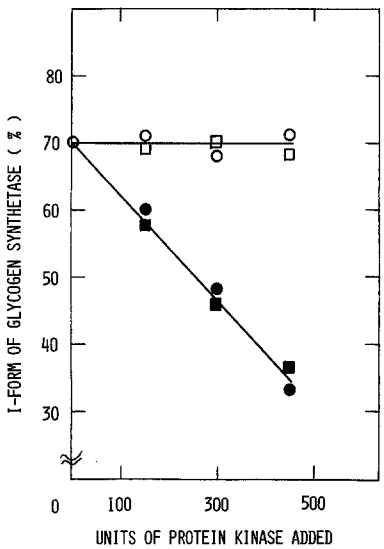

Fig. 2 *Inhibition of muscle glycogen synthetase by catalytic subunits of yeast and liver cyclic AMP-dependent protein kinases.* The reaction mixture (0.10 ml) initially contained 25 µg of bovine serum albumin, 1 µmole of Tris-Cl at pH 7.5, 0.75 µmole of magnesium acetate, 10 nmoles of ATP, 4 µg of rabbit skeletal muscle glycogen synthetase and catalytic subunit of either yeast or rat liver cyclic AMP-dependent protein kinase as indicated. After incubation for 10 min at 30°C, 0.05 ml of a solution containing 5 µmoles of Tris-Cl at pH 7.5, 1 µmole of EDTA, 1.0 mg of glycogen and 0.1 µmole of UDP-[U-^{14}C]glucose (150,000 cpm/µmole), was added and the mixture was incubated for additional 10 min at 30°C. Another reaction was carried out with 1 µmole of glucose 6-phosphate as an additional ingredient in order to assay the total activity (I- and D-forms) of glycogen synthetase. The reaction was finally terminated by the addition of 10 ml of 75% ethanol and radioactive glycogen produced was determined by the method previously described (5). Unit of protein kinase activity is defined in Table I. ●——● and ○——○ represent experimental values with yeast protein kinase in the presence and absence of ATP, respectively; ■——■ and □——□ with rat liver protein kinase in the presence and absence of ATP, respectively.

protein kinases were used, the inhibition of glycogen synthetase was dependent on the addition of cyclic AMP. Neither protein kinase, cyclic AMP nor ATP alone was active in the conversion of I-form to D-form of this enzyme.

By analogy to the mammalian phosphorylase system Fosset *et al.* have described two, active and inactive, forms of glycogen phosphorylase from yeast *(S. cerevisiae)*, and that the inactive form is converted to the active form in the presence of ATP and a specific phosphorylase kinase (18). Similarly, two interconvertible forms,

glucose 6-phosphate-dependent (D-form) and independent (I-form), of glycogen synthetase in yeast have been described by Rothman-Denes and Cabib (19). The conversion of I-form to D-form has been also proposed to be catalyzed by a protein kinase by Huang and Cabib (20). However, the direct involvement of cyclic AMP in the regulation of glycogen metabolism in this microorganism has not been so far reported. Nevertheless, these observations together with the experimental results presented in this paper imply that in eukaryotic microorganisms cyclic AMP may play roles in a similar manner to that described for mammalian tissues, and probably regulates glycogen metabolism through activation of the cyclic AMP-dependent protein kinase. The factors which trigger unidirectional conversion between active and inactive forms of various participating enzymes *in vivo* are also inevitable for further investigations.

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