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Isolation and properties of a protein kinase from rat liver microsomes

In a previous paper the isolation from rat liver cytosol of a protein kinase was reported[1]. In the present note the isolation of a protein kinase from rat liver microsomes is described whose properties, after partial purification, appear to be different from those of the cytosol enzyme.

The data reported in Table I clearly show that a saline extract from acetonic powders of twice-washed rat liver microsomes was able to catalyze the incorporation of ^{32}P from [γ-^{32}P]ATP into phosvitin. No incorporation was observed from $^{32}P_i$.

TABLE I

PROTEIN KINASE ACTIVITY IN MICROSOMES AND IN MICROSOMAL SUBFRACTIONS

Rat liver microsomes were prepared by centrifuging the 25 000 × *g* postmitochondrial supernatant of the 0.25 M sucrose liver homogenate at 105 000 × *g* for 1 h. The precipitated microsomes were washed twice in 20 times their volume of 0.25 M sucrose. Subfractionation of microsomes was achieved by layering the 0.25 M sucrose suspension of microsomes over 10 times its volume of 1.3 M sucrose and by centrifuging at 105 000 × *g* for 120 min. A well-packed pellet and a band not penetrating the 1.3 M sucrose were obtained and are referred to as the "heavy" and "light" microsomal fractions, respectively. Protein kinase was solubilized by extracting the microsomal subfractions or their acetonic powders with 10 times their volume of 0.1 M phosphate buffer (pH 6.8) containing 0.1 mM EDTA for 5 h at 2°. The insoluble material was discarded by centrifugation at 30 000 × *g*, and the clear supernatant was dialyzed 3 times in 100 times its volume of 0.05 M Tris–HCl buffer (pH 7.0) containing 0.1 mM EDTA. Protein kinase activity was tested following a procedure very similar to that already described for the cytosol enzyme[1]: 0.5 μmole of ATP containing 1–2 μC as [γ-^{32}P]ATP prepared according to GLYNN AND CHAPPEL[2], and 1 mg phosvitin prepared according to MECHAM AND OLCOTT[3] were present in the incubation medium. Incubation was started by addition of 2 mg of microsomal extract and carried out for 30 min at 37°. Controls without the addition of phosvitin were always carried through the whole procedure, and the resulting counts/min (never more than 20% of the radioactivity incorporated into phosvitin) were subtracted from the counts/min incorporated in the presence of phosvitin. RNA was determined according to MORSE AND CARTER[4].

Expt.	*^{32}P incorporated into phosvitin (counts/min per mg protein per h)*	*RNA (μg ribose/ mg protein)*
1. Unfractionated microsomes	1150	—
2. Subfractionated microsomes:		
"light" fraction	540	8.43
"heavy" fraction	1788	27.18

Moreover the repartition of such a protein kinase activity between the "heavy" and "light" microsomal subfractions closely paralleled that of RNA (Table I), suggesting that this enzyme is associated with the "rough" microsomes containing the ribosomes.

The microsomal protein kinase has been purified by precipitation at pH 4.7 followed by extraction of the precipitate with 0.1 M phosphate buffer (pH 7.0) and by fractionation with $(NH_4)_2SO_4$. The fraction that precipitated between 0.25 and 0.55 $(NH_4)_2SO_4$ saturation, retaining over 90% of the total activity, was dissolved in the minimal volume of 0.05 M Tris (pH 7.0) containing 0.1 mM EDTA and dialyzed 3 times against 50 times its volume of the same buffer. By this procedure the enzyme was purified about 10 times.

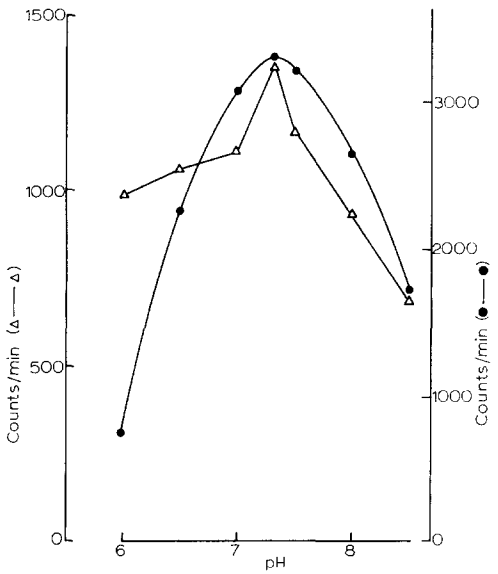

Fig. 1. Partially purified protein kinase was prepared as described in the text. The enzyme activity was tested as previously described[1]. pH 6.0–7.5 buffers were Tris acetate; pH 7.5–8.5 buffers were Tris–HCl. Incubation was started by the addition of the enzymes: 0.1 mg of cytosol protein kinase (●—●) and 0.5 mg of microsomal protein kinase (△—△), and lasted for 30 min at 37°.

The properties of the partially purified microsomal protein kinase were compared with those of the cytosol enzyme, purified to approximately the same extent by a very similar procedure, which has been already described[1]. These two enzyme preparations have been found to behave significantly differently in several respects: (1) Their respective curves of activity as a function of pH, reported in Fig. 1, are not superimposable, particularly in the range between 6 and 7: in fact the activity of cytosol enzyme was practically negligible at pH 6, while that of microsomal enzyme remained almost unchanged. (2) While the two apparent Michaelis constants with phosvitin for the microsomal and cytosol enzymes were similar (0.55 and 0.68 mg/ml, respectively), the two K_m values with casein were quite different (4.00 and 0.66 mg/ml, respectively). (3) Unlabeled GTP, which reduced the labeling of phosvitin by [^{32}P]ATP in the presence of the cytosol enzyme, had little or no effect in the presence of the microsomal enzyme. Such an inhibition is quite insensitive to arsenate which is known to be an inhibitor of nucleosidediphosphate kinase (EC 2.7.4.6) reaction[5]. (4) The cytosol protein kinase activity was strongly reduced when phosphoproteins partially dephosphorylated by spleen protein phosphatase, were used as substrate. On the contrary the microsomal enzyme maintained almost unchanged its activity towards the partially dephosphorylated substrates (Table II).

This latter property of microsomal protein kinase and its localization in the ribosomal structures suggest the possibility that the microsomal protein kinase might be responsible for the primary input of phosphate into newly synthesized protein molecules, while the cytosol enzyme might be only involved in the phosphate turnover of already phosphorylated phosphoproteins.

TABLE II PHOSPHORYLATION OF DEPHOSPHORYLATED PHOSPHOPROTEINS BY CYTOSOL AND MICROSOMAL PROTEINKINASES

Protein kinase activity was tested under the same conditions of Fig. 1: pH was 7.5. Hammarsten casein was from Merck. Partially dephosphorylated casein and phosvitin were prepared by submitting these two proteins to enzymatic dephosphorylation by calf spleen protein phosphatase, free of proteolytic activity, prepared according to SUNDARARAJAN AND SARMA[6]. For the preparation of dephosphorylated casein the procedure was very similar to that described by SUNDARARAJAN AND SARMA[6]. In the case of phosvitin, however, since this protein, unlike casein, is still soluble at pH 6 after dephosphorylation, it was recovered at the end of incubation by precipitation with 10% trichloroacetic acid, dissolved in water at neutral pH and dialyzed twice in water. The extent of dephosphorylation of casein and phosvitin, determined on the basis of their residual alkali-labile phosphate, was found to be 91 and 87%, respectively. The numbers in parentheses indicate the per cent variation of ^{32}P incorporation compared to that occurring with the phosphorylated protein.

Expt.	*^{32}P incorporated into phosphoprotein (nmoles/mg phosphoprotein)*	
	Microsomal protein kinase	*Cytosol protein kinase*
1. Phosvitin	39.5	51.9
Dephosphorylated phosvitin	42.5 (+7.5%)	6.8 (−86.9%)
2. Casein	17.8	32.9
Dephosphorylated casein	13.2 (−25.9%)	4.2 (−87.3%)

Experiments are now in progress in order to obtain evidence about the different nature of these two liver protein kinases not only in terms of their biological activity, but also of their chemical structure.

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