Short Communication

Phosphorylation of Initiation Factor 2 by Non-Phosphorylated Initiation Factor 2 α Kinase from Rabbit Reticulocytes

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Protein synthesis in reticulocytes and their lysates is controlled by the availability of heme.^{1~4)} In the absence of heme, protein synthesis is rapidly shut off after a lag in cell-free systems.^{3,4)} This cessation is mediated through the activation of initiation factor 2 α kinase (eIF-2 α kinase), which specifically phosphorylates the α subunit of reticulocyte initiation factor 2 (eIF-2 α).^{5~9)} This kinase is present in hemin-supplemented lysates in inactive form,⁷⁾ and is activated in the absence of added hemin or on treatment with sulfhydryl reagents such as *N*-ethylmaleimide (NEM).^{10,11)}

eIF-2 α Kinase autophosphorylates, and its kinase activity is proportional to the extent of autophosphorylation.^{7,11~13)} However, there are no direct evidence that the phosphorylation of eIF-2 α kinase is essential for its kinase activity. During studies of the phosphorylation of pig liver eIF-2 by eIF-2 α kinase treated with NEM,¹⁴⁾ we found that chymotrypsinogen inhibited the autophosphorylation of kinase, but did not affect the phosphorylation of pig liver eIF-2 α (lanes 1 and 2 in Fig. 1). Since the phosphorylation and dephosphorylation of eIF-2 α are related to the regulation of globin synthesis in hemin-deficient reticulocyte lysate,^{15~17)} we examined the phosphorylation of reticulocyte eIF-2 in the presence of chymotrypsinogen. Data similar to those for pig liver eIF-2 were obtained (lanes 3 and 4). To check whether phospho-

rylation-inhibited eIF-2 α kinase phosphorylates eIF-2 α , eIF-2 α kinase treated with NEM was incubated for 5 min at 30°C with chymotrypsinogen to inhibit autophosphorylation,

FIG. 1. Effect of Chymotrypsinogen on the Phosphorylation of eIF-2 α and NEM-treated eIF-2 α Kinase.

NEM-treated eIF-2 α kinase and reticulocyte eIF-2 were prepared as described elsewhere.¹⁸⁾ Pig liver eIF-2 was prepared from a high-salt extract of pig liver.¹⁴⁾ The kinase and pig liver eIF-2 were the same preparation as used before.¹⁴⁾ NEM-treated eIF-2 α kinase (1.8 μ g for lanes 1 and 2, and 3.3 μ g for lanes 3~5) was incubated with eIF-2 from pig liver (2.1 μ g, lanes 1 and 2) or reticulocytes (9.9 μ g, lanes 3 and 4) in the presence (lanes 2, 4, 5) or absence (lanes 1 and 3) of 5 μ g of chymotrypsinogen (Pharmacia Fine Chemicals) in a mixture containing [³²P]ATP (New England Nuclear Corp.). The total volume of the mixture was 25 μ l (lanes 1~4) or 20 μ l (lane 5). The incubation mixture in a final volume of 25 μ l contained 30 mM Tris-HCl (pH 7.4), 2 mM magnesium acetate, 3 mM mercaptoethanol, 0.1 mM EDTA, 0.2 mM [³²P]ATP (250 mCi/mmol) and 100 mM K⁺. After incubation for 5 min at 30°C, some samples (lanes 1~4) had 25 μ l of buffer A (62.5 mM Tris-HCl, pH 6.8, 2% sodium dodecyl sulfate, 1% glycerol and 5% mercaptoethanol) added, and the whole was boiled for 5 min. The other sample (lane 5) had 5 μ l of reticulocyte eIF-2 (9.9 μ g) added and was incubated for 5 min at 30°C and then boiled for 5 min after addition of 25 μ l of buffer A. All samples were analyzed by sodium dodecyl sulfate-polyacrylamide (10%) slab gel electrophoresis as described elsewhere.¹⁸⁾ An autoradiograph of the dried gel is shown, with migration positions of eIF-2 α kinase (*M*_r 80,000) and eIF-2 α (*M*_r 38,000). Chymo. stands for chymotrypsinogen.

and then reticulocyte eIF-2 was added and the whole was incubated for 5 min at 30°C. Analysis of the incubation mixture showed that non-phosphorylated eIF-2 α kinase phosphorylated reticulocyte eIF-2 α (lane 5).

These findings showed that autophosphorylation of the kinase is not essential for its kinase activity. But we do not know how chymotrypsinogen inhibits the autophosphorylation of the kinase but not the kinase activity.

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