CYCLIC AMP-INDEPENDENT GLYCOGEN SYNTHASE KINASE FROM RAT LIVER

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1. Introduction

The presence of cyclic AMP-independent casein/phosvitin kinases able to phosphorylate glycogen synthase I has been reported in rabbit skeletal muscle [1]. One of these kinases incorporated ⩽4 mol phosphate/mol 85 000 dalton subunit. Such phosphorylation led to a total I–D-form conversion of the enzyme.

Rat liver cytosol has been shown to contain two cyclic AMP-independent casein kinases which can be resolved by Sepharose 6B gel-filtration and display different catalytic activities towards casein fractions [2,3]. However, the physiological role of these hepatic casein kinases has not been established yet. On the other hand, in crude preparations of rat liver a cyclic AMP-independent glycogen synthase kinase activity has been indicated [4] but no characterization of such activity has been reported. The aim of this work was to study the ability of a purified cyclic AMP-independent casein kinase from rat liver to phosphorylate and inactivate glycogen synthase I.

2. Experimental

2.1. *Materials*

Casein was Hammarsten quality from Merck; histone IIA was from Sigma Chemical Co. DEAE-cellulose (DE-32) and phosphocellulose (P-11) were from Whatman; Sepharose 4B was from Pharmacia Fine Chemicals. Casein coupling to Sepharose 4B was according to [5]. [γ-^{32}P]ATP was prepared as in [6].

2.2. *Enzymes*

Cyclic AMP-independent casein kinase (CK-1) has been purified by a method combining chromatography on phosphocellulose and casein–Sepharose 4B. The initial steps of the purification were similar to those used for the rabbit muscle enzymes [1]. The casein kinase eluted from the phosphocellulose column with 0.65 M KCl was dialyzed overnight against 50 mM Tris/Cl buffer (pH 7.5) containing 1 mM dithiothreitol, 0.1 mM phenylmethylsulfonylfluoride and 5% glycerol (buffer A) and applied to a (4 × 10 cm) casein–Sepharose 4B column equilibrated with the same buffer. After washing the column with 250 ml buffer A a 500 ml linear gradient of 0–1 M KCl in the same buffer was applied. Under these conditions the enzyme eluted at ~0.55 M KCl. The casein kinase preparation was then 2–3-fold diluted with buffer A and applied to a (1.1 × 2 cm) phosphocellulose column equilibrated with the same buffer. After washing with equilibrium buffer containing 0.2 M KCl the casein kinase was eluted by raising KCl to 1 M. Finally, it was dialyzed overnight against buffer A and kept stored at −20°C. The specific activity of this enzyme preparation was 200 units/mg protein.

A crude preparation of cyclic AMP-dependent protein kinase (peak I) having spec. act. 1 unit/mg protein was obtained according to [7] by chromatography on DEAE-cellulose of the histone kinase peak excluded from phosphocellulose.

Cyclic AMP-dependent protein kinase inhibitor protein was purified up to the trichloroacetic acid precipitation step according to [8].

Homogenous I-form glycogen synthase was prepared from rabbit muscle according to [9].

2.3. *Assays*

Kinase activity was assayed at 30°C as in [1]. One unit of kinase activity is defined as the amount of enzyme that catalyzes the transfer of 1 nmol ^{32}P from [γ-^{32}P]ATP to casein or histone per minute under the standard assay conditions.

Glycogen synthase phosphorylation and I–D-form conversion studies were at 30°C in parallel assays in the presence and absence of labelled ATP (200–400 cpm/pmol), respectively. In any case the assay mixture (0.2 ml) contained 25 mM β-glycerol-phosphate (pH 7.0), 1.5 mM EDTA, 1.5 mM EGTA, 1 mM dithiothreitol, 0.125 mM ATP, 8 mM Mg-acetate, 0.02 mg/ml glycogen, 0.09 mg/ml glycogen synthase I and 0.7 units/ml casein kinase. At indicated times 20 μl aliquots were removed and assayed either for ^{32}P incorporation or glycogen synthase independence ratio (*RI*) change, respectively.

The amount of ^{32}P incorporated into protein was measured after separation from the unreacted [γ-^{32}P]-ATP by ITLC chromatography according to [10].

Glycogen synthase activity was determined in the absence and presence of 7.2 mM glucose-6-P according to [11]. The *RI* is defined as the activity in the absence of glucose-6-P divided by the activity in its presence, the result being multiplied by 100.

3. Results

Rat liver cytoplasm CK-1 kinase preparation was free of endogenous protein substrates and phosphorylated preferentially the acidic protein casein (fig.1). The rate of phosphorylation of histone was <2% of that of casein. The activity of the kinase on either substrate assayed was not stimulated by 2×10^{-5} M cyclic AMP. Identical cyclic AMP concentrations stimulated ~3-fold histone phosphorylation by a crude preparation of rat liver cyclic AMP-dependent protein kinase (fig.2). Cyclic AMP-

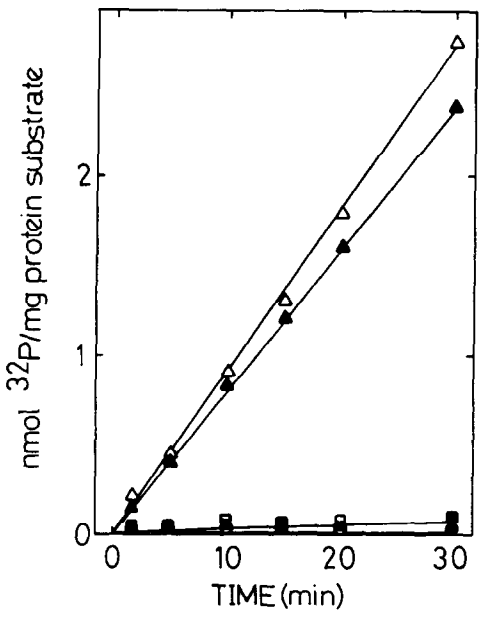

Fig.1. Substrate preference of CK-1 kinase. (○,△,□) no cyclic AMP; (●,▲,■) 2×10^{-5} M cyclic AMP; (○,●) no exogenous substrate, (△,▲) 4 mg/ml casein, (□,■) 4 mg/ml histone. CK-1 kinase concentration was 0.4 units/ml.

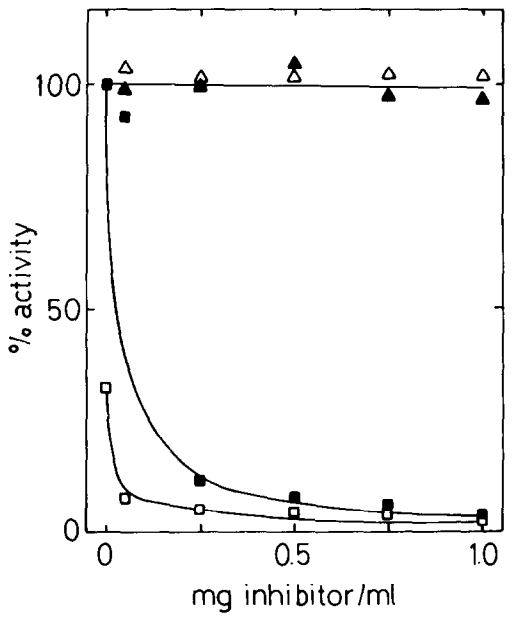

Fig.2. Effect of cyclic AMP-dependent protein kinase inhibitor protein on CK-1 kinase (casein as substrate) (△,▲) and cyclic AMP-dependent protein kinase (histone as substrate) (□,■). (△,□) no cyclic AMP; (▲,■) 2×10^{-5} M cyclic AMP. Data refer to the activity of each kinase in the presence of 2×10^{-5} M cyclic AMP and absence of inhibitor protein. Kinase was 0.4 units/ml in each case.

dependent protein kinase inhibitor protein did not cause any inhibition on the CK-1 kinase activity at ≤1 mg/ml (fig.2). Under these conditions the activity of the cyclic AMP-dependent protein kinase was strongly inhibited both in the absence and in the presence of cyclic AMP. Thus, CK-1 kinase is clearly different from the cyclic AMP-dependent protein kinase or its catalytic subunit.

Besides casein, CK-1 kinase also phosphorylated homogenous I-form glycogen synthase (fig.3). The ^{32}P incorporation correlated with a decrease in the *RI*, rendering a glycogen synthase with a very high degree of dependence on glucose-6-P. The maximum extent of phosphorylation achieved was 3.6 ± 0.4 mol ^{32}P/mol 85 000 dalton subunit, that promoted a decrease in the *RI* from 83 + 3–15 + 4. Data are mean ± SD of 4 experiments. No significant ^{32}P incorporation or *RI* change was observed in the absence of added CK-1 kinase (fig.3). Cyclic AMP did not stimulate the phosphorylation of glycogen synthase by CK-1 kinase (table 1). In fact, the ^{32}P incorporation in the presence of cyclic AMP was slightly lower than in its absence.

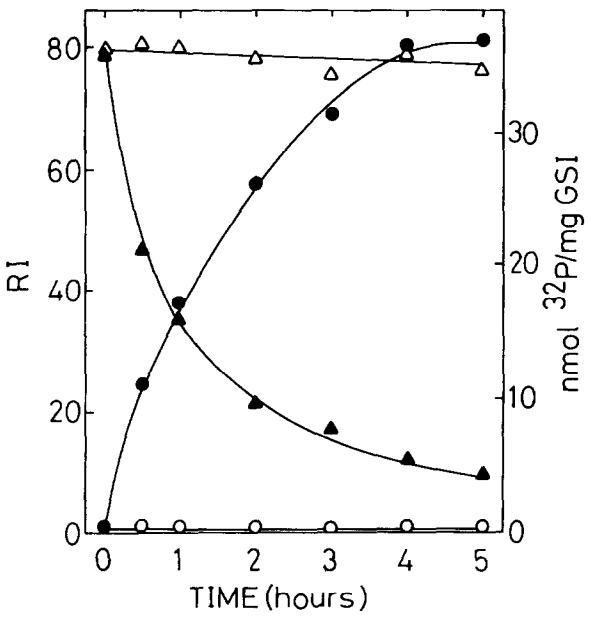

Fig.3. ^{32}P-incorporation (○,●) and *RI* change (△,▲) of I-form glycogen synthase (GSI) in the absence (○,△) and presence (●,▲) of added CK-1 kinase. Details are in section 2.

4. Discussion

Evidence has been accumulated indicating the presence in rabbit muscle of protein kinases that can phosphorylate I-form glycogen synthase in a cyclic AMP-independent manner [1,12]. One of such kinases can totally phosphorylate and convert the glycogen synthase I into D [1]. High concentrations of cyclic AMP-independent glycogen synthase kinases have been recently detected in a variety of tissues [4] where they account for a considerable percentage of the total glycogen synthase kinase activity.

The results reported here demonstrate that rat liver contains a cyclic AMP-independent glycogen synthase kinase that can incorporate ~3.6 mol ^{32}P/mol 85 000 dalton subunit of glycogen synthase. This phosphorylation results in a great decrease in its *RI*. It has to be stressed that the phosphorylation and thus the I–D-form conversion of glycogen synthase was due to the CK-1 kinase and not to the presence of any endogenous kinase in the glycogen synthase preparation [12] since no significant ^{32}P incorporation or *RI* change were observed in the absence of added CK-1 kinase.

It would be very interesting to compare the degree

Table 1
Effect of cyclic AMP (2×10^{-5} M) on the phosphorylation of I-form glycogen synthase by CK-1 kinase

Time (h)	mol ^{32}P/mol 85 000 dalton subunit −cAMP	+cAMP	% incorporation +cAMP/−cAMP
0.5	1.21	0.79	65
1	1.58	0.98	62
3	2.50	1.70	68
5	3.57	2.88	81

of phosphorylation of glycogen synthase I achieved by CK-1 kinase and purified rat liver cyclic AMP-dependent protein kinase. In fact, the presence of cyclic AMP-dependent protein kinases able to phosphorylate and convert glycogen synthase I into D have been reported in rat and bovine liver [13,14]. However no details are so far available on the total phosphate incorporated and *RI* change achieved on glycogen synthase by these enzymes. Nonetheless, CK-1 kinase is different from the cyclic AMP-dependent protein kinase or its catalytic subunit in that:

(i) It phosphorylates casein but poorly histone;
(ii) Its activity on either substrate assayed is not stimulated by cyclic AMP;
(iii) It is not inhibited by the cyclic AMP-dependent protein kinase inhibitor protein.

On the other hand, the extent of phosphorylation and I–D-form conversion achieved by CK-1 kinase are comparable to those observed with the rabbit muscle cyclic AMP-independent glycogen synthase kinase [1].

Whether CK-1 kinase corresponds to one of the so-called 'TS' and 'S' casein kinases reported in rat liver [3] or it represents a different enzyme is not clear yet since no molecular or kinetic data are available for comparison.

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