

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 \times 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 \times 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-

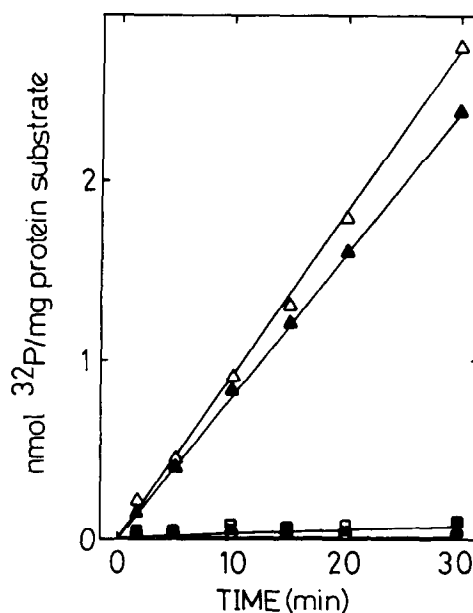


Fig.1. Substrate preference of CK-1 kinase. (\circ, Δ, \square) no cyclic AMP; ($\bullet, \blacktriangle, \blacksquare$) 2×10^{-5} M cyclic AMP; (\circ, \bullet) no exogenous substrate, (Δ, \blacktriangle) 4 mg/ml casein, (\square, \blacksquare) 4 mg/ml histone. CK-1 kinase concentration was 0.4 units/ml.

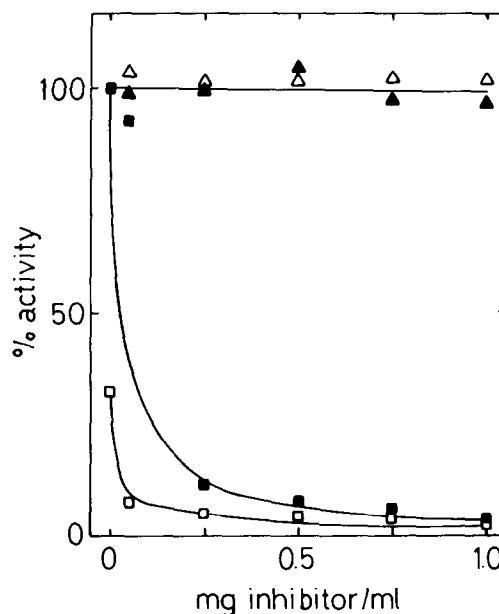


Fig.2. Effect of cyclic AMP-dependent protein kinase inhibitor protein on CK-1 kinase (casein as substrate) (Δ, \blacktriangle) and cyclic AMP-dependent protein kinase (histone as substrate) (\square, \blacksquare). (Δ, \square) no cyclic AMP; ($\blacktriangle, \blacksquare$) 2×10^{-5} M cyclic AMP. 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 \pm 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.

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

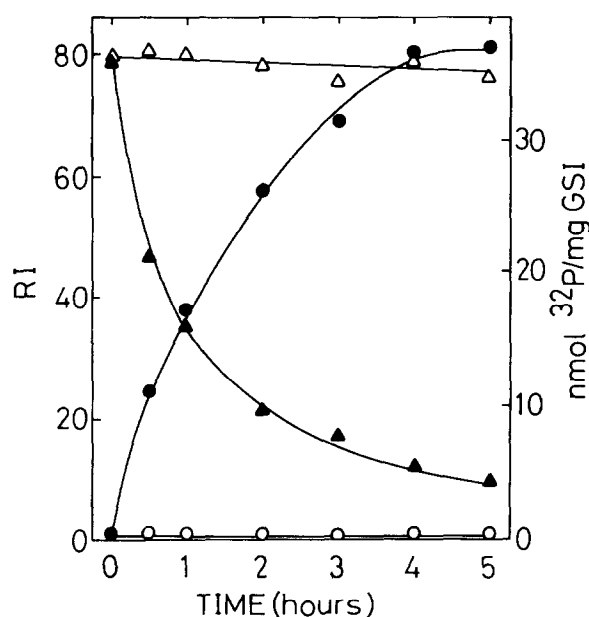


Fig.3. ^{32}P -incorporation (○,●) and *RI* change (Δ,▲) of I-form glycogen synthase (GSI) in the absence (○,Δ) and presence (●,▲) of added CK-1 kinase. Details are in section 2.

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	mol ^{32}P /mol 85 000 dalton subunit +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 *R/I* 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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