INSULIN SENSITIVITY OF LIVER GLYCOGEN SYNTHASE $b$ INTO $a$ CONVERSION*

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Summary

Liver glycogen synthase $b$ phosphatase, chromatographically separable from phosphorylase $a$ phosphatase, is decreased in 48-hour alloxan diabetic rats. The phosphatase activities are measured in an in vitro system using exogenous isolated phospho-enzyme as substrates with added phosphatases. Synthase and phosphorylase phosphatases were shown to have differential catalytic properties by their reactivity in the presence of Pi, the heat-stable inhibitor of phosphorylase phosphatase and after incubation with added cAMP-dependent protein kinase.

Introduction

At the present time, liver glycogen synthesis in vivo is believed mediated by the active form of glycogen synthase (synthase $a$ or $I$) resulting from hormonal or metabolite regulation of phosphoprotein phosphatase-catalyzed dephosphorylation of physiologically inactive synthase $b$ (synthase $D$)\(^1,2\). Recent evidence suggests the liver to contain either several substrate specific phosphoprotein phosphatases\(^3-5\) or interconvertible forms or a single phosphoprotein phosphatase\(^6-10\) with synthase or phosphorylase-specific phosphatase activity possibly expressed in response to changes in large molecular weight modulators of the phosphatase\(^11-19\).

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The present study was designed to determine the insulin-specific sensitivity of liver synthase phosphatase as measured in vitro by synthase $b$ into $a$ conversion activity\(^20-21\). The results of this investigation show that an isolated synthase $b$ phosphatase, which is chromatographically distinct from phosphorylase $a$ phosphatase is decreased in the liver of 48-hour alloxan diabetic rats and that insulin pre-treatment of the diabetic animals results in a return to a near normal level of the isolated synthase $b$ into $a$ conversion activity.

Materials and Methods

**Materials**

Radioactive compounds UDP-$^{14}$C-glucose and $^{14}$C-glucose-1-P were obtained from New England Nuclear Corp., Boston. All other compounds and reagents were of the best quality available and were obtained from either Sigma Chemical Co. or Fisher Scientific, St. Louis, Mo. The DEAE-cellulose (DE-52, Whatman):Sephadex G-25 (Pharmacia) columns were prepared on a 3:2 weight basis, respectively, to improve column flow rates. Proteins were determined by the method of BRADFORD\(^22\) and were comparable to the LOWRY method\(^23\). Glycogen was determined by the anthrone method\(^24\).

**Animals**

Male, Sprague-Dawley rats (175–200 g) were made diabetic with a single I.P. injection of freshly prepared alloxan in 0.9% NaCl (100–150 mg/Kg body weight) following an overnight fast. Forty-eight hours after treatment, only rats clearly diabetic were used for the study as determined with tail-vein blood using Dextrostix.
(Ames Co.). Quantitative blood glucose determinations were done when the animals were killed using the kit (no. 510) from Sigma Chemical Co. The blood glucose values were (mg/ml): (a) normal, 0.9–1.2; (b) diabetic, 4.0–8.0; (c) insulin-treated diabetic (4 units I.P. 3 hours before killing), 1.5–2.5.

Assay of enzymes
Glycogen synthase was assayed by the filter paper method whereby $^{14}$C-glucose is transferred from UDP-$^{14}$C-glucose to glycogen acceptor. Enzyme fractions were added to a mixture (final volume 0.25 ml) containing 13.75 μmole glycylglycine, pH 7.4, 1.25 mg rabbit liver glycogen, 0.5 μmole UDP-glucose (with 11,000–14,000 cpm UDP-$^{14}$C-glucose) with 2.5 μmole of either glucose-6-P for synthase b or Na$_2$SO$_4$ for synthase a. After incubation at 37° for 4 minutes, 0.1 ml of the mixture was adsorbed to filter paper (Whatman ET-31) and processed for counting as described for this method. A unit of synthase activity is the amount of enzyme which transfers 1.0 μmole of glucose from UDP-glucose to glycogen at the end of the assay time.

The assay for phosphorylase activity was the transfer of $^{14}$C-glucose from $^{14}$C-glucose-1-P to glycogen acceptor. The enzyme preparation was added to a mixture containing 12.5 μmole glycylglycine, pH 6.1, 1.25 mg rabbit liver glycogen, 5.0 μmole glucose-1-P (with 21,000 cpm $^{14}$C-glucose-1-P) 5.0 μmole KF and 0.25 μmole AMP (final volume, 0.25 ml). After 4 minutes at 37°, 0.1 ml of the mixture was adsorbed to Whatman ET-31 filter paper and processed for counting as described for this method. A unit of phosphorylase is the amount of enzyme which transfers 1.0 μmole of glucose from glucose-1-P to glycogen at the end of the assay time.

Exogenous substrate enzymes
Liver glycogen synthase b and phosphorylase a were prepared from normal, fed animals for use as exogenous substrates for the phosphatase reactions described herein. Livers were quickly removed, chilled in and blended with three volumes of ice-cold 0.25 M sucrose containing 0.1 M glycylglycine, pH 7.4, 0.1 M KF and 0.02 M 2-mercaptoethanol. An 8,000 × g extract was prepared by centrifugation of the homogenate (10 min) and packed glycogen pellets were obtained by centrifugation of the 8,000 × g fraction at 50,000 × g for 60 min. The glycogen pellets, which contained 80–90% of synthase and phosphorylase measured relative to the enzyme amounts in the 8,000 × g extract, were resuspended in cold, 0.1 M glycylglycine, pH 7.4, containing 0.1 M KF, 0.02 M 2-mercaptoethanol as well as 20% (v:v) glycerol (Buffer A) and centrifuged again at 50,000 × g for 60 min. The ‘wash’ centrifugation results in a loss of about 6–10% of synthase and phosphorylase activities (average range of 10 preparations). The packed, glycogen pellets could be stored frozen as such without loss of enzyme activities upon thawing at 4° similar to that reported for rabbit liver synthase.

To free synthase and phosphorylase from glycogen before chromatographic separation of the enzymes, glycogen phosphorolysis was done using endogenous phosphorylase by adding, per g wet weight of packed glycogen pellet (either freshly prepared or frozen), 10 ml of a buffer composed of 0.1 M glycylglycine, pH 6.9, containing 0.1 M KF, 0.02 M 2-mercaptoethanol, 20% (v:v) glycerol and 0.05 M KH$_2$PO$_4$. The suspension of glycogen was dialyzed against the same buffer for 24–36 hours at room temperature, without loss of enzyme activities, during which time the glycogen content decreased from 11.0–12.0 mg/ml to 0.7–0.8 mg/ml and the solution changed appearance from milk-white to clear. The solution was then dialyzed overnight in the cold against Buffer A to remove KH$_2$PO$_4$. Insoluble material, occasionally present after the dialysis in the cold, was removed by centrifugation at 10,000 × g (10 min) without an effect on the levels of either synthase or phosphorylase. The cleared solution (20–30 ml with 0.8–1.1 mg protein/ml; 1.0–3.0 units synthase b/ml and 25.0–35.0 units phosphorylase a/ml) was adsorbed to a DEAE-cellulose (DE-52):Sephadex G-25 column (2.2×24 cm) equilibrated with Buffer A. After collecting the initial 280 nm absorbing material in a single fraction, phosphorylase a and synthase b were eluted from the column, in 5.0 ml fractions, with 0.1 M and 0.2 M NaCl-supplemented Buffer A, respectively. All of the fractions with either synthase or phosphorylase were separately