IN VITRO ACTIVATION OF LEUKOCYTE GLYCOGEN SYNTHETASE

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SUMMARY: The activity of leukocyte glycogen synthetase in a freshly prepared homogenate is almost completely in the b form. Incubation of the homogenate at 30°C caused a time dependent increase in the activity measured in the absence of G-6-P (b to a conversion). The K_a for G-6-P decreased from 0.7 to 0.01 mM. Freezing of the homogenate resulted in a complete loss of the capacity for activation. These results demonstrate that glycogen synthetase from leukocytes of normal human subjects can be converted in vitro to a form, which is almost independent of G-6-P for activity.

Glycogen synthetase has been shown to exist in two interconvertible forms (designated I and D or a and b respectively) in liver (1, 2), muscle (3) and adipose tissue (4). In these tissues activation (b to a conversion) can be brought about in vivo by administration of various hormones or glucose (5, 6), or in vitro by incubation of crude or partially purified tissue homogenates (7, 1).

In polymorphonuclear leukocytes from healthy human subjects it has not previously been possible to demonstrate a glucose-6-P (G-6-P) independent activity (a form), either in a freshly prepared homogenate (8 - 10) or following in vitro activation (8), although such activation could be obtained with some leukocyte preparations from diabetic patients on insulin therapy (11). Activation of leukocyte glycogen synthetase from normal subjects has recently been demonstrated by incubation of a cell suspension with glucose, but only when the cells had previously been "starved" by incubation without substrate for 2 h (12).

In this report we show that incubation of a leukocyte homogenate at 30°C results in a time-dependent conversion of glycogen synthetase from a

form which is inactive in the absence of G-6-P, to one almost completely independent of this ligand.

METHODS

Blood was obtained from healthy volunteers. Leukocytes were separated by differential sedimentation with 3% dextran (13). The leukocyte pellet was suspended in 0.05 M Tris-HCl buffer, pH 7.5,to a concentration of 7.5 x 10^7 cells/ml, consisting of about 90% polymorphonuclears, and sonicated for 30 sec. in a Branson sonifier at 8 Amp. Results were not affected when sonication time was varied from 15 sec. to 2 min. All procedures were carried out at 0^o to 4^oC.

Glycogen synthetase activity was assayed by incorporation of UDP-(U-^{14}C) glucose into glycogen. The reaction mixture contained: 1.6 mM UDP-^{14}C glucose, 2% rabbit liver glycogen, 80 mM glycyl glycine buffer, pH 7.5, 10 µl of enzyme solution and G-6-P as indicated in the legends. Incubation was carried out for 10 min at 30^oC with constant shaking. The reaction was terminated by addition of 2 ml of diazyme solution, and the incorporation of ^{14}C glucose into glycogen was measured according to De Wulf et al. (14).

MATERIALS

UDP-glucose, rabbit liver glycogen G-6-P, nucleotides and glycylglycine were purchased from Sigma Chemical Co. UDP-(U-^{14}C) glucose was obtained from the Radiochemical Centre, Amersham, England. Amberlite resins were purchased from BDH Chemicals, Ltd., Poole, England. Diazyme^R (Aspergillus niger amyloglucosidase) was a product of Miles Chemical Co., Clifton, N.J. Diazyme solution was prepared according to De Wulf et al. (14). Sephadex G-25 (medium) was purchased from Pharmacia Fine Chemicals AB, Uppsala, Sweden.

RESULTS

Incubation of a leukocyte homogenate at 30^oC resulted in an increase

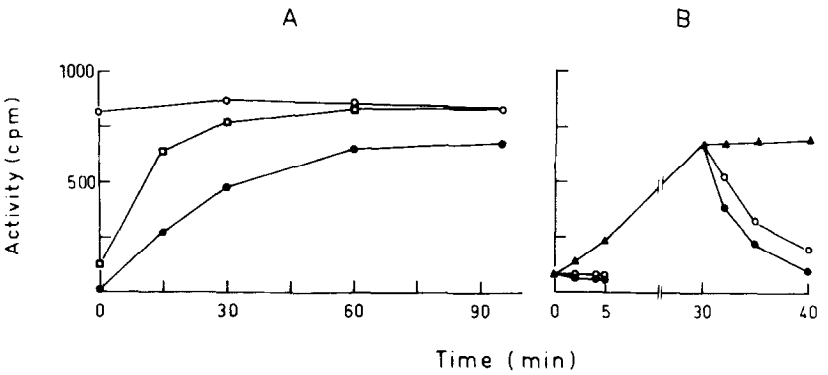

Fig.1A Activation of glycogen synthetase.

The homogenate was pre-incubated at 30°C and at the times indicated 0.010 ml was added to the assay mixture. G-6-P concentration in the assay mixture was: 0 (●), 0.2 mM (□) and 10 mM (o).

Fig.1B Inactivation of glycogen synthetase by ATP-Mg.

A leukocyte homogenate containing 4 mM Mg acetate was incubated at 30°C for 40 min. At 0 and 30 min the following additions were made: none (▲), 2 mM ATP (o) or 2 mM ATP and 0.5 mM dibutyril 3'5' Cyclic AMP (●). Samples were withdrawn at the times indicated and assayed in the presence of 0.1 mM G-6-P.

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in the activity of glycogen synthetase, measured without added G-6-P, from zero to about 2/3 of the total activity (as measured with 10 mM G-6-P) within 30 min. When the assay was carried out with 0.1 to 0.3 mM G-6-P, the increase in activity was from 5 to 10% respectively to 100% of the activity with 10 mM G-6-P (Fig.1).

Addition of ATP-Mg to the homogenate after 30 min of activation caused a prompt decline of the activity to baseline levels, which was accelerated by 3'5'-dibutyryl cyclic AMP (Fig.1B). The addition of ATP-Mg at zero time caused only a slight decrease in activity, indicating that the activity in a freshly prepared homogenate is indeed almost totally in the b form.

The effect of the method of cell disruption on the total activity of glycogen synthetase and on the activity of the phosphatase catalyzing the b

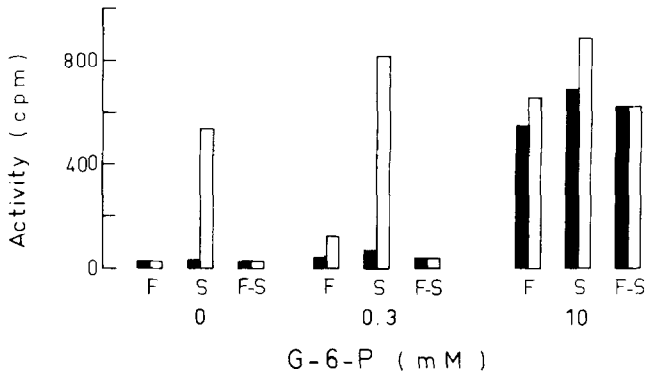

Fig.2 Effect of freezing on activation of glycogen synthetase.

A leukocyte suspension was treated by freezing and thawing (F), sonication,as described in Methods (S), or sonication followed by freezing (F-S). Activity was measured at the indicated concentrations of G-6-P before (dark column) or after (open column) activation for 30 min.

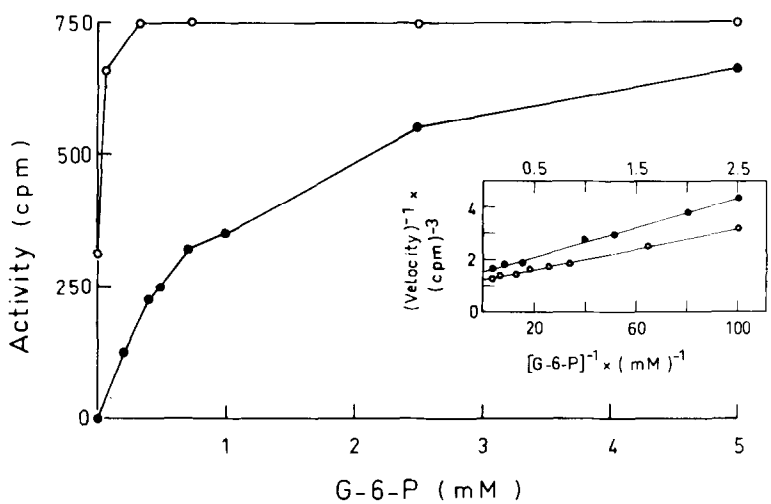

Fig.3 Effect of G-6-P on the activity of glycogen synthetase.

Activation was carried out as described in Fig.1. The non activated (•) and activated (o) preparations were filtered through a Sephadex G-25 column at 4°C prior to the assay, to remove endogenous G-6-P and other low molecular weight metabolites. Assays were performed with 0.025 ml of filtrate and variable G-6-P concentrations. The inset shows a double reciprocal plot of the same data.

to a conversion is shown in Fig.2. The total synthetase activity was similar irrespective of whether the cells had been disrupted by sonication or freezing and thawing. On the other hand, the activity of the synthetase

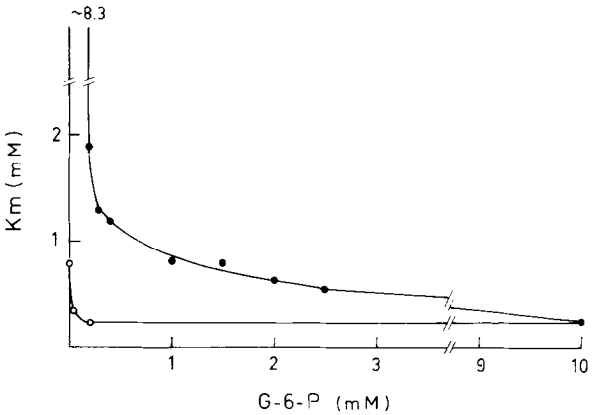

Fig.4 Effect of G-6-P concentration on K_m for UDP-glucose.

K_m was determined with variable UDP-glucose concentrations at the indicated concentrations of G-6-P before (●) and after (o) activation.

phosphatase was extremely small in the homogenate prepared by freezing and thawing. Freezing of a preparation obtained by sonication resulted in a complete loss of the capacity for activation, whereas the same treatment had no effect on a liver homogenate (not shown).

The effect of in vitro activation on the affinity of the enzyme for G-6-P is shown in Fig.3. The K_a for G-6-P was decreased by activation from 0.70 mM to about 0.01 mM. Fig.4 shows the effect of G-6-P on the K_m for UDP-glucose before and after activation. When the enzyme was saturated with respect to G-6-P, the K_m for UDP-glucose was 0.25 mM in both preparations. This K_m value was obtained with G-6-P concentrations of 10 mM and 0.2 mM respectively.

DISCUSSION

It has been shown that the changes in the kinetic parameters of leukocyte glycogen synthetase from normal subjects upon in vitro activation are similar to those previously described for the liver enzyme (2). These results clearly demonstrate that in vitro activation of glycogen synthetase can be obtained in leukocytes from normal subjects, and is not confined only to cells from diabetic patients (11) and from various rodents (15).

In accord with the results of other investigators (8-10), we have also found that almost all the activity in a freshly prepared homogenate is in the b form. This situation is similar to that obtained with the mouse liver enzyme which, in an untreated animal, is also mostly in the b form (16).

The extreme sensitivity of the activating system to freezing seems to be unique for the leukocyte enzyme, and further work will be needed to determine whether it is possible to protect the phosphatase against this type of inactivation.

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