FACTORS AFFECTING THE ACTIVITY OF MUSCLE GLYCOGEN SYNTHETASE[*]

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Glycogen synthetase (UDP glucose: α-1,4-glucan α-4-glucosyltransferase) of rat muscle has been found to be activated by glucose-6-phosphate (Leloir et al., 1959). Further investigation indicated that the enzyme exists in two forms: one, glucose-6-phosphate dependent (D); the other independent of this factor (I) (Rosell-Perez, Villar-Palasi and Larner, 1962). It was established that a conversion of the I to the D form occurs in the presence of ATP and apparently involves a phosphorylation at the protein level. The reverse reaction, D to I conversion, has also been demonstrated. A loss of the phosphate bound to the D form of the enzyme coincides with this transformation (Traut and Lipmann, 1963; Friedman and Larner, 1963).

This note reports the existence of alternative methods for I to D conversion of muscle glycogen synthetase. The mechanisms involved in this system appear to be very similar to those regulating muscle phosphorylase b kinase activity (Krebs, Graves and Fischer, 1959; Meyer, 1962).

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Methods

Glycogen synthetase was assayed at 30° C by following the production of UDP in an incubation mixture that contained 0.005M UDP glucose, 1 % glycogen, 0.05M tris-HCl pH 7.5, 0.0025M EDTA and the enzyme in a final volume of 0.04 ml. The conversions between the two forms of the enzyme were expressed as changes in the relative glucose-6-phosphate independence defined by the ratio: activity in the absence of glucose-6-phosphate (I) / activity in the presence of 0.01M glucose-6-phosphate (I + D).

Phosphorylase b kinase activity was assayed by the method of Krebs, Graves and Fischer (1959), using crystalline rabbit muscle phosphorylase b as the substrate. Kinase activation was indicated by an increase in activity measured at pH 6.8.

The glycogen synthetase preparations used in this work were purified by the following procedure. A cold neutral extract of fresh rat muscle, containing 0.004M EDTA, was adjusted to pH 5.1 with 1N acetic acid. After centrifugation, the precipitate was resuspended in EDTA, neutralized and centrifuged to remove insoluble material. This acid precipitate fraction was then brought to 40 % of saturation by the addition of a saturated ammonium sulphate solution. After centrifugation, the pellet obtained was taken up in a small volume of 0.05M tris-HCl, pH 7.3, containing 0.004M EDTA, and then dialyzed overnight against the same buffer.

To achieve maximum activity, it was necessary to preincubate all preparations for 30 minutes at 30° C in the presence of 0.05M mercaptoethanol. This treatment did not cause a significant change in the relative glucose-6-phosphate independence of the enzyme.

Results

Figure 1 shows the effects of Ca^{++}, ATP-Mg^{++} and trypsin on the relative glucose-6-phosphate independence of glycogen synthetase.

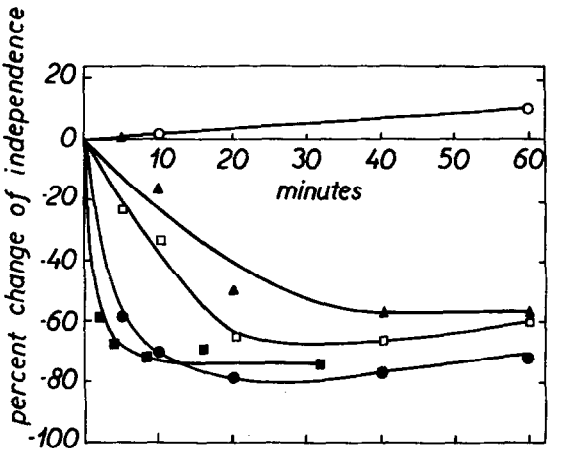

Fig.1: Effects of Ca^{++}, ATP-Mg^{++} and trypsin on the relative glucose-6-phosphate independence of glycogen synthetase. A concentrated acid precipitate fraction from rat muscle was pretreated with mercaptoethanol (see "Methods") and then incubated at 30° C with 0.07M tris-HCl, pH 7.3, in the presence of the following additions: 0.01M ATP and 0.02M $MgCl_2$ ▲——▲ ; 0.01M ATP, 0.02M $MgCl_2$ and 0.0005M cyclic 3'5'-AMP □——□ ; 0.01M $CaCl_2$ ●——● ; no additions ○——○ . Aliquots were taken from the incubation mixtures at the indicated times and diluted five fold in cold 0.04M tris-HCl, pH 7.5, containing 0.02M EDTA. The incubation with trypsin ■——■ contained: 0.02M tris-HCl, pH 7.5, 0.01M EDTA and 0.02 mg/ml of crystalline trypsin. This reaction was stopped by the addition of crystalline soybean trypsin inhibitor to a final concentration of 0.2 mg/ml.

These effects appear to be independent of each other. Assays for glucose-6-phosphate and ATP (Kornberg, 1950), before and after incubation with Ca^{++}, were negative. The Ca^{++} effect could be demonstrated in purified enzyme preparations which were not responsive to ATP-Mg^{++} and vice versa. Cyclic 3'5' AMP produced a significant stimulation of the ATP-Mg^{++} reaction, but was ineffective on incubation with Ca^{++}. Trypsin was able to reduce the glucose-6-phosphate independence of the synthetase at all stages of purification and at concentrations of EDTA which completely blocked the other reactions. The enzymes, modified by the above incubations, showed similar independence

ratios before and after precipitation with ammonium sulphate and dialysis.

The change induced by Ca^{++} on the activity of glycogen synthetase has been studied in some detail. EDTA and other Ca^{++} chelators blocked but did not reverse the reaction. There was an appreciable Ca^{++} effect at all pH's between 6.5 and 8.5 but above pH 7.5 a Ca^{++} catalyzed loss of the total activity occurred. At a concentration of 0.02M, Sr^{++} and Ba^{++} were 92 and 14 % as effective, respectively, as Ca^{++}; Zn^{++}, Mg^{++} and Mn^{++} were ineffective.

As in the case of phosphorylase b kinase (Meyer, 1962), the Ca^{++} effect requires the presence of a protein factor which can be separated from the glycogen synthetase in the ultracentrifuge. A dilute acid precipitate fraction from rat muscle was clarified by freezing and thawing. The insoluble material was removed by low speed centrifugation and the supernatant centrifuged 2.5 hours at 100,000 x g. The pellet thus obtained was resuspended and centrifuged again. The resulting precipitate contained most of the glycogen synthetase, as well as phosphorylase b kinase, but was essentially free of the factors required for the action of Ca^{++} on both enzymes. Readdition of the supernatant of the first 100,000 x g centrifugation, or of unpurified acid precipitate, restored the action of Ca^{++}.

The "Ca^{++} factor" in the 100,000 x g supernatant was heat labile, non-dialyzable and destroyed by trypsin. No effect of Ca^{++} was observed unless all three components (factor, glycogen synthetase and Ca^{++}) were incubated together. Preliminary experiments have indicated that the factors responsible for the Ca^{++} effects on glycogen synthetase and phosphorylase b kinase co-chromatograph on DEAE-cellulose and Sephadex G-200 columns.

Winegrad and Shanes (1962) demonstrated that Ca^{++} enters the cell during excitation of cardiac muscle fibers. It has been postulated that in some regions of the cell the Ca^{++}

concentration becomes high enough to inhibit the relaxing system and thereby initiates contraction. Krebs and Fischer (1963) have suggested that this Ca^{++} influx might activate phosphorylase b kinase. These effects would be complemented by a simultaneous Ca^{++} dependent conversion of glycogen synthetase to a less active form.

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