# MUSCLE GLYCOGEN SYNTHASE IN VIVO STATE:

## Effects of insulin administration on the chemical and kinetic properties of the purified enzyme

P. J. ROACH[a], M. ROSELL-PEREZ[b] and J. LARNER[c]

*Department of Pharmacology, University of Virginia School of Medicine, Charlottesville, Virginia 22903, USA*

Received 16 May 1977

### 1. Introduction

Control of the phosphorylation state of rabbit-muscle glycogen synthase (UDPglucose: glycogen 4-α-glucosyltransferase, EC 2.4.1.11) by the action of hormones such as insulin and epinephrine [1,2] is considered to be an important feature of the endocrine regulation of the activity of this enzyme. The control of activity follows from the fact that the kinetic properties of glycogen synthase are strongly dependent on the phosphorylation state of the enzyme [3,4], a higher phosphorylation state correlating with lower activity under most conditions. In previous work, we investigated the relationship between enzyme kinetic properties of synthase and the alkali-labile phosphate content of the enzyme [3,4]. Enzymes differing in phosphorylation state were obtained by incubation of partially purified enzyme containing kinase(s) with ATP, cyclic-AMP and $Mg^{2+}$ for different lengths of time.

These experiments indicated a smooth, monotonic dependence of properties on phosphate content. Therefore, we wished to observe whether endogenous or native enzyme purified without deliberate conversion (that is, phosphorylation or de-phosphorylation) had properties that correlated with our earlier findings. Also, we wished to know whether the effects of perturbation of synthase in vivo by insulin were transmitted to the properties of glycogen synthase in a manner consistent with our experiments in vitro. We report here that the properties of the enzyme purified from control animals and from animals treated with glucose plus insulin fell close to our previous results with the isolated enzyme.

### 2. Materials and methods

#### 2.1. *Assays for glycogen synthase activity*

Standard assays for glycogen synthase were performed as in Thomas et al. [5]. The *%I* activity is defined as the ratio of enzyme activity in the absence of glucose 6-P to that in the presence of 7.2 mM glucose 6-P, expressed as a percentage. A unit of enzyme activity is defined as 1 μmol of UDPglucose incorporated into glycogen per minute. Enzyme assays to determine kinetic parameters were similar, and details have been described [3].

#### 2.2. *Purification of glycogen synthase*

Glycogen synthase was purified from each of several rabbits, treated as below, using the published procedure for the *D* (glucose-6-P dependent, phosphorylated) form of the enzyme [6]. No conversion step was, however, included and the enzyme was eluted from the DEAE-cellulose column with buffer containing 0.25 M NaCl.

[a]Present address: Laboratorio di Biochimica, Via A. Volta 4, Università di Pisa, 56100 Pisa, Italy

[b]Deceased, to whom this paper is dedicated

[c]Supported in part by USPHS Grant AM 14334 and the University of Virginia Diabetes Research Center Grant AM 17042

*Abbreviations:* $S_{0.5}$, substrate concentration required for half-maximal activity, $M_{0.5}$, modifier concentration required for half-maximal effect

2.3. *Treatment of rabbits*

Male rabbits of 2 to 4 kg weight were subjected to various treatments as below, before being killed by the injection into the marginal vein of the ear of 3 ml pentobarbital.

*Controls:* Two rabbits were killed without treatment (giving enzyme samples C1 and C2.

*Glucose/insulin:* Two rabbits received intraperitoneal injections of glucose (20% w/v) to give final doses of 2 g/kg body weight. After either 4 min (I1) or 10 min (I2), 0.66 ml of 1 mg/ml insulin (Novo Pork-Lot MC-S-821506) was injected into the marginal vein of the ear. The animals were killed 10 min after the insulin injections.

2.4. *Other methods*

Determinations of the alkali-labile phosphate contents of purified glycogen synthase samples were carried out as described by Smith et al. [7]. Protein was estimated by the method of Lowry et al. [8].

## 3. Results

3.1. *Enzyme purification and the relationship of %I activity with hormone treatment*

Glycogen synthase was purified from the four rabbits treated as described above. It is evident from table 1 that at the stage of the crude muscle extracts, the dependency of the enzyme activity on glucose 6-P (*%I* activity) correlated with most current theories of insulin action. Insulin treatment after a previous glucose injection caused a change in *%I* activity from 48% or 56% to 63%.

We hoped in these experiments to retain the hormone-induced alterations of synthase properties during the purification of the enzyme. Therefore, $F^-$ was present in the initial stages of the purification to inhibit phosphatase activity, and later the normal incubation with ATP, cyclic-AMP and $Mg^{2+}$ was omitted to prevent further phosphorylation. Examination of table 1 indicates that in fact there were some changes in the *%I* activity during purification. The control and the glucose/insulin samples did, however, maintain a difference in *%I* activity throughout purification. The final *%I* activities of the controls were 40% and 42% and of the insulin treated animals 55% and 51%.

3.2. *Chemical characterization of enzyme samples*

A summary of the properties of the various enzyme samples is shown in table 2. Previously, values for the specific activity of purified glycogen synthase in this laboratory were between 30 and 40 units/mg [3,6]. The values obtained here are, therefore, low. The results of polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (not shown) did indicate slightly elevated levels of impurities but insufficient to account for a 50% reduction in specific activity. A possible explanation for the lower specific activities could, therefore, be the presence of inactive glycogen synthase, but this is not proven. The alkali-labile phosphate contents of the two insulin samples

Table 1
Glucose 6-P dependency (*%I* activity) as a function of glycogen[a] synthase purification

| Sample | *%I* Activity | | | |
|---|---|---|---|---|
| | Tissue extract | After EtOH precipitation | Before Sepharose 4B chromatography | Final |
| C1 | 48 | 48 | 33 | 40 |
| C2 | 56 | 43 | 27 | 42 |
| I1 | 63 | 56 | 39 | 55 |
| I2 | 63 | 53 | 35 | 51 |

[a]Standard assays, in the presence and absence of 7.2 mM glucose 6-P were performed as described in Materials and methods. The details of the treatments for the different samples is also given in the text; enzymes C1 and C2 came from control rabbits, enzymes I1 and I2 from rabbits treated with glucose then insulin

Table 2
Characterization of glycogen synthase samples

| Sample[a] | Alkali-labile[b] phosphate (P/subunit) | %I Activity | Specific[c] activity (units/mg) | UDPglucose[d] varied | | Glucose 6-P[e] varied | |
|---|---|---|---|---|---|---|---|
| | | | | $S_{0.5}$ (mM) | Hill slope | $M_{0.5}$ (mM) | Hill slope |
| C1 | 1.31 | 40 | 9.3 | 5.5 | 0.86 | 0.037 | 0.93 |
| C2 | 1.34 | 42 | 12 | 6.6 | 0.76 | 0.020 | 1.00 |
| I1 | 0.98 | 55 | 23 | 4.6 | 0.80 | 0.013 | 0.88 |
| I2 | 0.75 | 51 | 20 | 5.1 | 0.78 | 0.014 | 0.83 |

[a]Samples as identified in the text, with C for control, I for glucose/insulin treated rabbits.
[b]Average of triplicates, except for C1 which was done in duplicate, and assuming a subunit molecular weight of 85 000 [6]
[c]Determined by standard assay in the presence of 7.2 mM glucose 6-P
[d]Assays as in Materials and methods, with $S_{0.5}$ determined from Hill plots
[e]Assays as in Materials and methods, with 0.2 mM UDPglucose; $M_{0.5}$ determined from Hill plots

were less than those of the controls, consistent with the idea that insulin action favors production of the dephosphorylated form of glycogen synthase.

### 3.3. *Kinetic characterization of enzyme samples*

When the substrate, UDPglucose, concentration was varied, deviations from Michaelis–Menten kinetics were observed, similar to those found previously [3]. Hill plots had slopes in the range 0.76 to 0.86, average 0.80, compared with a value of 0.79 found before [3]. Values for $S_{0.5}$ obtained from Hill plots are shown in table 2. Activation by glucose 6-P for samples C1, C2, and I1 followed essentially hyperbolic activation kinetics although one sample, I2, had a rather lower Hill slope (0.83).

### 3.4. *Correlation of enzymic properties with previous results for phosphorylation in vitro*

As described above, previous studies of glycogen synthase related various enzymic properties with the

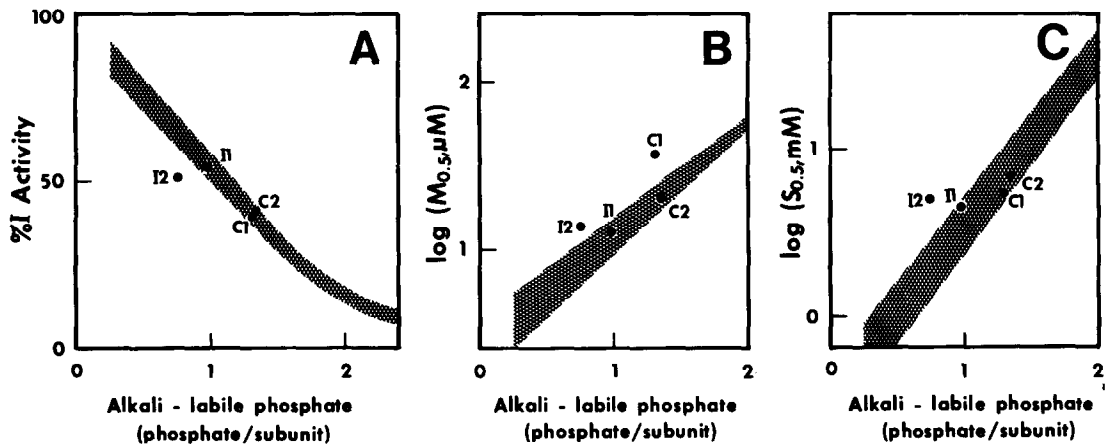


Fig.1. Comparison of data for current glycogen synthase samples with earlier results with the enzyme. Enzyme kinetic parameters; (A), %I activity; (B) $M_{0.5}$ for glucose 6-P; and (C), $S_{0.5}$ for UDPglucose obtained in the present studies are shown compared with data from earlier work in this laboratory (see text). In particular, the correlation between kinetic properties and phosphate content has been illustrated. The shaded areas represent the range of values from previous studies. The results for different enzymes are identified by the numbering described in Materials and methods, and the exact values for the parameters are to be found in table 2.

phosphorylation state achieved by incubation of the enzyme at a stage of partial purification, with ATP, cyclic AMP and $Mg^{2+}$ [3]. The data for the enzyme samples described here are compared with these earlier results in fig.1. Three parameters were used: *%I* activity, $S_{0.5}$ for UDPglucose, and $M_{0.5}$ for glucose 6-P. The relationship between *%I* activity and phosphate content was that predicted from in vitro studies for enzymes C1, C2, and I1, and sample I2 was close to the predicted range (fig.1A). With regard to glucose 6-P activation, both control and insulin samples were close to the predicted range (fig.1B) as were the values for the $S_{0.5}$ for UDPglucose (Fig.1C). We note that enzyme I2 was always the most aberrant.

## 4. Discussion

The first point of interest is the lack of constancy of the *%I* activities of the enzymes during purification. All samples decreased in *%I* activity subsequent to the first ethanol precipitation but regained *%I* activity after the final chromatography step. Thus, the overall changes in *%I* activity were not very large and the removal of soluble modifiers is a possible explanation for these changes.

In previous work, we had detailed the relationship between various kinetic properties of glycogen synthase and its degree of phosphorylation as obtained by incubations of the enzyme in vitro. For the enzymes obtained here from control rabbits and glucose/insulin treated rabbits, the data fitted quite well on the earlier plots of kinetic parameters versus phosphorylation state. This would tend to suggest that under some conditions, the relationship between enzymic characteristics and phosphate content found in vitro may be similar to that in vivo. Further, the sense of the insulin action, namely dephosphorylation, fits with current views of insulin control of glycogen metabolism. This dephosphorylation correlates with an increased *%I* activity, decreased $S_{0.5}$ for UDPglucose, and decreased $M_{0.5}$ for glucose 6-P. What is demonstrated here for the first time is that glycogen synthase in the basal state in vivo contains 1–2 mol of phosphate per subunit. Thus, the role of the covalent control by hormones would appear to be to modify the covalent phosphate content of the enzyme from a basal state to a new state. This is a different situation from the case of phosphorylase, where the interconversion entails the removal or addition of one phosphate per subunit. The implications of the control of glycogen synthase by multiple phosphorylation have been recently discussed [9,10].

## References

[1] Larner, J. and Villar-Palasi, C. (1971) Curr. Top. Cell. Regul. 3, 195–236.
[2] Stalmans, W. and Hers, H. G. (1973) in: The Enzymes, 3rd edn (Boyer, P. D. ed) Vol. 9, pp. 309–361, Academic Press, New York.
[3] Roach, P. J., Takeda, Y. and Larner, J. (1976) J. Biol. Chem. 251, 1913–1919.
[4] Roach, P. J. and Larner, J. (1976) J. Biol. Chem. 251, 1920–1925.
[5] Thomas, J. A., Schlender, K. K. and Larner, J. (1968) Anal. Biochem. 25, 486–499.
[6] Takeda, Y., Brewer, H. B. and Larner, J. (1975) J. Biol. Chem. 250, 8943–8950.
[7] Smith, C. H., Brown, N. E. and Larner, J. (1971) Biochim. Biophys. Acta 242, 81–88.
[8] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[9] Roach, P. J. and Larner, J. (1976) Trends in Biochemical Sciences, 1, 110–112.
[10] Roach, P. J. and Larner, J. (1977) Mol. Cell. Biochem. (In press).