THE COMBINED EFFECT OF GLYCOGEN AND ATP ON THE D TO I CONVERSION OF GLYCOGEN SYNTHETASE

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SUMMARY

The effects of ATP and glycogen on the D to I conversion of glycogen synthetase have been investigated in a gelfiltrate of a 17000 x g supernatant from human polymorphonuclear leukocytes. It was found, that 2 mM ATP barely inhibited the D to I conversion at low glycogen concentrations, whereas the inhibition increased substantially at higher glycogen concentrations. The possibility that this effect is part of the control mechanism of the enzyme is discussed.

Glycogen synthetase exists in most tissues in two interconvertible forms, synthetase D and synthetase I. Conversion of the D form to the I form is catalyzed by synthetase D phosphatase. Gilboe and Nuttall have shown, that the D to I conversion of glycogen synthetase is inhibited by physiological concentrations of ATP in muscle (1) and liver (2); in muscle extracts glycogen enhanced the inhibition by ATP. In contrast to this, Kato and Bishop (3) found no effect of ATP on their highly purified muscle glycogen synthetase phosphatase. This communication deals with the effect of physiological ATP-concentrations at increasing concentrations of glycogen on the D to I conversion of leukocyte glycogen synthetase.

MATERIALS AND METHODS

Experimental procedure.

Human polymorphonuclear leukocytes were isolated as outlined in a previous publication (4). 10^9 cells (~ 1 gram) were suspended in 1 ml preincubation buffer (Tris-HCl 50 mM, dithiothreitol 1 mM, pH 7.4), sonicated, and centrifuged at 17000 x g at 4° for 15 min. The supernatant was filtered through a column of Sephadex G-50 Fine, which had been equilibrated in preincubation buffer, and the effluent was incubated at 25° for 30 min. At intervals the D to I conversion of glycogen synthetase was followed. 150 µl samples were withdrawn and mixed with 100 µl of ice-cold dilution buffer (Tris-HCl 50 mM, EDTA 40 mM, NaF 50 mM, dithiothreitol 2 mM, pH 7.4) and placed in an ice-bath. Shortly afterwards each sample was filtered through 2 ml columns of Sephadex G-50 Fine, which had been equilibrated in dilution buffer, and assayed for enzyme activity and protein content. The last Sephadex filtration reduced the amount of low molecular weight metabolites to less than 0.1% of the concentration originally found in the sample.

Leukocyte glycogen was prepared essentially by a method already described for the purification of glycogen synthetase (5). The glycogen was filtered through a Sephadex G-50 column equilibrated in distilled water, heated at 50° C for 1 hour to destroy adhering enzymes, and lyophilized.

Analytical procedures.

Glycogen synthetase activity was determined with the method of Thomas et al. (6), using 4 mM Uridine diphosphate U-^{14}C-glucose (specific activity 2-3 x 10^5 cpm/µmole); I-activity and total activity were measured with 10 mM Na_2SO_4 and 6.7 mM glucose-6-phosphate, respectively, in the reaction mixture. 1 mUnit enzyme activity incorporates 1 nanomole glucose into glycogen per min. at 30°.

TABLE 1

Glycogen concentration	0,1% (n = 9)	0,25% (n = 9)	0,5% (n = 6)
No additions	0,171 ± 0,022	0,146 ± 0,022	0,081 ± 0,017
2 mM ATP	0,163 ± 0,019	0,085 ± 0,012	0,034 ± 0,010
Degree of inhibition	5%	42%	58%

Table 1. The effect of 2 mM ATP on the D to I conversion of glycogen synthetase at three different concentrations of glycogen.
The rates of conversion are given as mUnits of I-activity formed per min. during the interval from 10 to 30 min., where the reaction is fairly linear. Results are given as mean ± s.e.m.

Glycogen was determined as glucose using the hexokinase assay after 2 1/4 hours hydrolysis in 0.6 N HCl at 100°. Protein was determined by a Lowry method; samples were diluted 100 times before measurement to avoid interference by Tris and EDTA (7).

CHEMICALS

Chemicals were obtained from Boehringer and Sigma and used without further purification with the exception of rabbit liver glycogen (Boehringer grade II), which was treated with a mixed ion exchange resin (Amberlite MH-3) before use. Radioactive uridine diphosphate glucose was obtained from Amersham.

RESULTS AND DISCUSSION

During a recent series of experiments on the D to I conversion of glycogen synthetase in gelfiltrates of homogenates from human polymorphonuclear leukocytes, we found rather conflicting results regarding the effect of ATP on the reaction. In some experiments we found an

inhibition of the reaction whereas others showed no effect or even a small activation by a physiological concentration of ATP (2 mM). The effect of ATP became reproducible when we varied a second parameter, the concentration of glycogen in the incubation.

Table I shows a series of experiments where the concentration of glycogen in the incubation medium has been adjusted to the indicated values with commercial glycogen. It can be seen that the inhibition caused by 2 mM ATP increases from 5% at a concentration of 0,1% glycogen to 58% at 0,5% glycogen. Since glycogen itself is an inhibitor of the conversion, a further increase in glycogen suppressed the reaction almost totally, both in the presence and absence of ATP. Principally, the same results were obtained in a few experiments substituting leukocyte glycogen for the commercial product. It should be mentioned that added ATP is not degraded during the incubation period and that a dose-response relationship can be obtained by varying ATP from 1 mM to 4 mM.

Fig. 1 shows that 1 mM glucose-6-phosphate is better able to overcome the inhibition by ATP at low concentrations than at high concentrations of glycogen, whereas glucose (5,5 mM) has no effect on the reaction.

These results might indicate that glycogen plays a dual role in the control of the D to I conversion of glycogen synthetase, both as an inhibitor and as a prerequisite for obtaining inhibition by ATP. The glycogen concentration in whole cells decreases from 0.8% to 0.2% during incubation in a medium not containing glucose (4) and a similar variation can be expected to take place in vivo. Since the ATP concentration in the cells is 2 mM and probably does not fluctuate very much, the suggested control mechanism would be more flexible than one based on the constant inhibition of the D to I conversion by ATP.

The effect of glucose-6-phosphate was obtained with concentrations 5 to 10-fold the concentrations found in whole cells. Experiments with

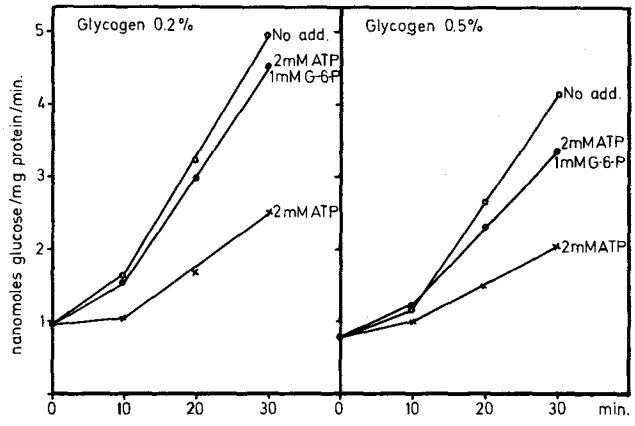

Fig. 1 The effect of 1 mM glucose-6-phosphate on the inhibition of the D to I conversion of glycogen synthetase by 2 mM ATP; experiments with 2 different glycogen concentrations are shown.

physiological concentrations of 0,1 and 0,2 mM glucose-6-phosphate failed to show any effect.

The molecular basis of the observed phenomena remains unresolved. One possibility is that glycogen synthetase phosphatase exists in two states, one at low concentrations of glycogen which is not affected by ATP, and one at high concentrations of glycogen which is inhibited by ATP. Another possibility is, that glycogen synthetase D exhibits conformational changes in the presence of ATP as suggested by Gilboe and Nuttall (2) and that these conformational changes are somehow modified by glycogen and glucose-6-phosphate. Since ATP is not inhibitory at low glycogen concentrations, however, it seems more likely that glycogen elicits the conformational change rather than ATP.

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